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The Biology of *Giardia* spp.

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OVERVIEW

Giardia is a binucleate flagellated protozoan parasite that causes intestinal infection in mammals, birds, reptiles, and amphibians. It was one of the first protozoans to be described when van Leeuwenhoek discovered the trophozoites in 1681 in his own diarrheal stool (77):

> "My excrement being so thin, I was . . . persuaded to examine it . . . wherein I have sometimes also seen animalcules a-moving very prettily; some of 'em a bit bigger, others a bit less, than a blood-globule, but all of the one and the same make; their bodies were somewhat longer than broad and their belly, which was flatlike, furnished with sundry little paws, wherewith they made such a stir in the clear medium, and among the globules, that you might e'en fancy you saw a pissabed running up against a wall; and albeit they made a quick motion with their paws, yet for all that they made but small progress."

Lambl described the genus *Giardia* in greater detail in 1859 (195); as a result, the human species was named after him. Despite its early discovery, *Giardia* only recently became universally accepted as a human pathogen because *Giardia* infections are noninvasive and frequently asymptomatic. However, the finding of severe giardiasis in patients with hypogammaglobulinemia, the occurrence of water-

borne outbreaks of diarrhea, and the fulfillment of Koch's postulates in experimental human infections (240) have confirmed its association with human disease. It is the most common protozoan isolated from human stool specimens (116) and is a common cause of diarrhea in humans as well as in dogs and cats (188).

Giardia has been placed in the phylum Sarcomastigophora and class Zoomastigophorea, along with the other flagellated human parasites, *Trypanosoma*, *Leishmania*, *Trichomonas*, and *Diemtamoeba*, and has been classified in the Order Diplomonadida (201). However, the sequence of its small-subunit rRNA is markedly different from those of all other protozoans, and on the basis of the small-subunit rRNA sequence, it has been proposed as the most primitive eukaryote (296). Morphological criteria have been used to divide the genus *Giardia* into three species (114). *Giardia agilis* infects amphibians; *G. muris* infects rodents, birds, and reptiles; and *G. lamblia* infects a variety of mammals, including humans, as well as birds and reptiles. *G. lamblia* (also called *G. intestinalis* and *G. duodenalis*) has more recently been divided into several species on the basis of more subtle differences seen by electron microscopy (see below). This review focuses primarily on *G. lamblia*, not only because it is the species associated with human disease, but also because it is the only species that has been axenized, so it has been used for all the in vitro studies. Mice infected with *G. muris* provide a useful model for studying the immune response to *Giardia* infection, and this model will be discussed in the section on the immune response to giardiasis.

Giardia has a simple life cycle consisting of an infective cyst and a vegetative trophozoite. The cyst is relatively resistant to environmental desiccation as well as to gastric acid of the host stomach. After a cyst is ingested, it excysts in the small intestine to form two trophozoites. The trophozoite divides by binary fission in the small intestine and is responsible for the symptoms of giardiasis. Some of the trophozoites are induced to encyst, and the cycle is completed when the cysts are passed in the feces and ingested by another host.

TROPHOZOITE STRUCTURE

The *G. lamblia* trophozoite is approximately 10 to 12 μm long and 5 to 7 μm wide. The cytoskeleton consists of a funis (body); a median body; a concave surface on the anterior two-thirds of the ventral surface, variously referred as a sucking disk, striated disk, or ventral disk; and four pairs of flagella (Fig. 1). A Golgi apparatus has been reported in encysting trophozoites (discussed in the encystation-excystation section) (267). Other distinct organelles, including two nuclei that appear identical and lysosomal vacuoles, are present. Glycogen and ribosomal granules are present in the cytoplasm. In concert with its classification as a primitive eukaryote, many of the usual eukaryotic organelles, including mitochondria, peroxisomes, smooth endoplasmic reticulum, and nucleoli, have not been identified.

Flagella

The four pairs of flagella (anterior, caudal, posterior, and ventral) emerge from basal bodies near the midline and anteroventral to the nuclei. Nine pairs of microtubules are symmetrically placed around the outer part of each flagellum, with two microtubules in the middle. Although flagellar motion is associated with motility, the function of the flagella has not been rigorously investigated. If a selective inhibitor of flagellar function could be identified, the role of the flagella could be more rigorously investigated. The suggestion that the flagella are involved in adherence (160) is discussed below.

Ventral Disk and Attachment

The site for attachment to the intestinal mucosa of the host is provided by the ventral disk, which probably acts as a suction cup in attachment to the intestine. Alternative mechanisms include a model in which flagellar motion provides the necessary hydrodynamic force to allow attachment (160) and one in which receptor-mediated interactions are used (103). Discussion of the following studies on the structure, chemistry, and physiology of the disk will take into account these potential mechanisms.

The ventral disk is a concave structure taking up most of the anterior two-thirds of the ventral surface of the trophozoite. It is surrounded by a rim which contacts and actually disrupts the architecture of the intestinal microvilli (159); surrounding the rim is a ventrolateral flange (Fig. 1). The disk appears to be fairly rigid and is made of microtubules, cross bridges attached to the microtubules, and unique structures called microribbons that are perpendicular to both the microtubules and the cross bridges (Fig. 1). The microtubules contain tubulin, and the microribbons contain giardins (256). The giardins are a set of *Giardia*-specific proteins found only in the ventral disk and range in size from approximately 29 to 38 kDa (67, 68, 161, 162, 256). Up to 23 distinct giardins have been identified by using two-dimensional electrophoresis, but some of these forms are the same in their N-terminal sequences and antigenically, which suggests that some of the giardins may be derived by different posttranslational modifications of the same protein (256). At least two distinct categories of giardins have been cloned and sequenced: α- and β-giardins. α_1-Giardin is 33.8 kDa and is predicted to be predominantly alpha-helical in its secondary structure (256). α_2-Giardin has been cloned and is similar to α_1-giardin in its DNA and amino acid sequences (81 and 77%, respectively) (15). β-Giardin (29.4 kDa) is also predicted to be alpha-helical in structure and has a heptad repeat consistent with a coiled-coil structure, as is found in myosin and tropomyosin (162). β-Giardin shows no DNA or amino acid similarity to α-giardin. The two published sequences (6, 22) differ only at G+C-rich regions and in the putative initiation site. The predicted amino acid sequences for an internal portion are very different, but it is possible that these differences are due to sequencing errors rather than being true differences. Of interest, the genomic clone (6) contains an ATG 4 bases 5′ to the beginning of the cDNA sequence reported (22). It is 33 bases 5′ to the other ATG and is in the same reading frame; it is more likely to be the true site of initiation, but this has not been confirmed by primer extension or S1 nuclease protection experiments.

In contrast to the rigid structure of the disk itself, the rim around the disk demonstrates flexibility in its attachment to intestinal mucosa (95) and appears to disrupt the intestinal mucosal surface during attachment (95, 250, 260). Immunoelectron-microscopic studies have localized the contractile proteins, actin, α-actinin, myosin, and tropomyosin to the rim (112). Therefore, the rim of the ventral disk appears to have contractile properties and to be closely involved in the attachment process. The attachment depends on active metabolism and is inhibited by oxygen, reduced temperature, reduced cysteine concentration (123, 127), and phar-

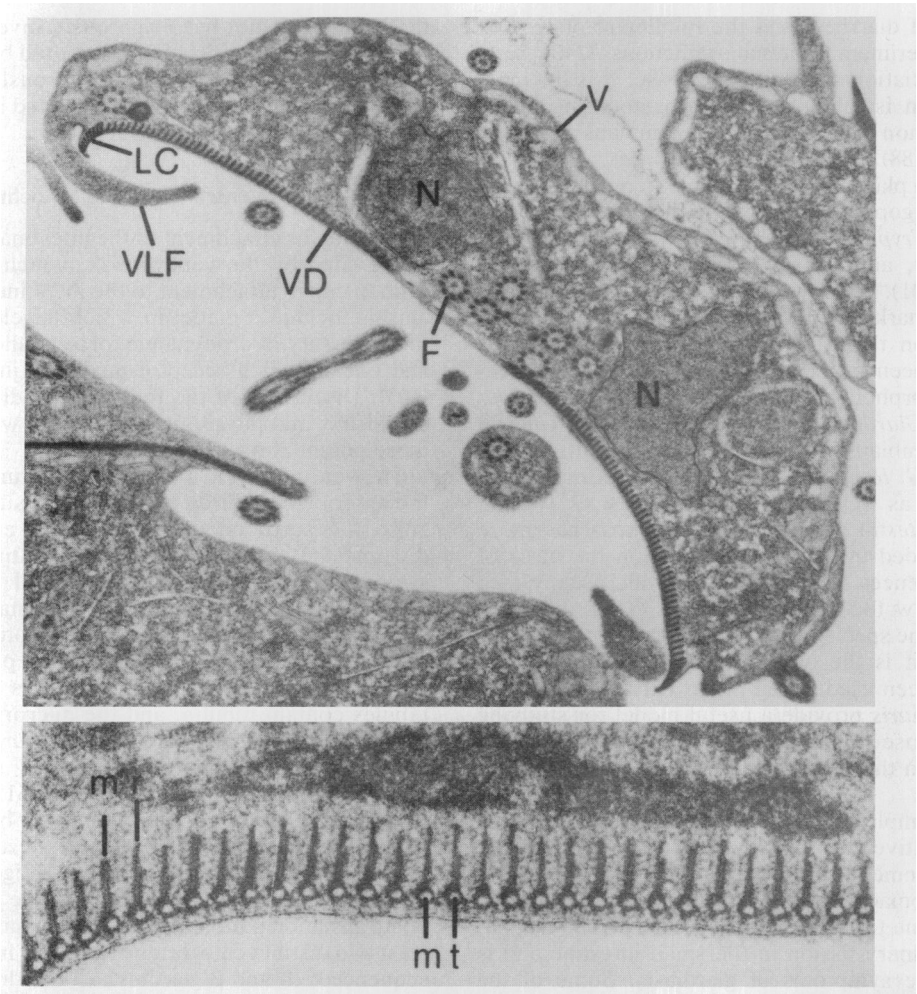


FIG. 1. Cross-sectional transmission electron micrograph of a *G. lamblia* trophozoite. LC, lateral crest; VLF, ventrolateral flange; VD, ventral disk; N, nucleus; F, flagella; V, vacuole. In the lower panel, a higher magnification shows the microtubules (mt) and microribbons (mr). Adapted with permission (256).

macologic agents with activity against *Giardia* spp., including metronidazole, furazolidone (70), and the benzimidazoles such as mebendazole (84). Benzimidazoles are believed to act by targeting β-tubulin (84), which is present in microtubules and flagella. They inhibit adherence by *Giardia* spp. with no apparent effect on flagellar function, suggesting the possibility that the flagellar tubulin lacks a binding site for benzamidazoles (84). Disk and flagellar tubulins are antigenically different (69, 307), providing further evidence that the disk and flagella contain distinct tubulins. The loss of adherence despite intact flagellar function indicates that flagellar function alone is not sufficient for attachment.

The surface of *Giardia* trophozoites contains a lectin (carbohydrate-binding moiety) that binds to mannose-glucose, and lectin-mediated receptor binding has been proposed as an alternative method of intestinal attachment (200). Trophozoites have been shown to bind to mammalian erythrocytes by this lectin binding; however, all sides of the trophozoite bind erythrocytes equally well (103). This lack of binding specificity for the ventral surface suggests that if lectin-mediated binding is part of the attachment process, it is a secondary rather than a primary mechanism of attachment. In addition, adherence to glass cannot be based on receptor-mediated binding. Thus, it appears most likely that *Giardia* trophozoites attach to the intestinal wall mechanically by means of suction generated by the ventral disk. The ability of *G. psittaci* (see below) to adhere despite the partial absence of the ventrolateral flange is inconsistent with the hydrodynamic model (91). In addition, the rapid reversal of adherence by the benzimidazoles despite apparently normal flagellar function (84), as well as adherence despite the absence of flagellar movement (112), indicates that flagella are not important in adherence.

Median Body

The median body is a structure in the midline and dorsal to the caudal flagella. It is unique to *Giardia* spp., and its morphology has been used to distinguish different *Giardia* spp. (114). *G. lamblia* trophozoites usually contain two median bodies which are shaped like claw hammers. The function of the median body is unknown, but the finding that antigiardin (69) and antitubulin (112) antibodies localize to the median body suggest that it may be functionally related to the ventral disk.

Golgi Apparatus

A structure fulfilling at last some of the criteria of a Golgi apparatus was initially reported during the analysis of in vitro encystation (267). Immunoelectron-microscopic studies revealed that encystation-specific antigens were localized in the Golgi apparatus. These antigens are transported to the forming cyst wall by encystation-specific vesicles (see below). In contrast, the constitutively expressed proteins, acid phosphatase and the cysteine-rich protein TSA 417 (126), are transported to their lysosomal and surface locations, respectively, by different routes. This suggests that the Golgi apparatus can sort proteins for transport to different locations. Golgi apparatuses have not yet been reported in nonencysting trophozoites, but the evidence for protein sorting makes it likely that all trophozoites contain them.

Vacuoles

Cysts and trophozoites contain ovoid vacuoles 0.1 to 0.4 μm in diameter that are adjacent to the plasmalemma (Fig. 1). In trophozoites they are found along the dorsal and ventral surfaces, but not in the region of the ventral disk. These vacuoles contain a variety of hydrolase activities, including acid phosphatase (109, 205), DNase, RNase, cysteine (thiol dependent), and thiol-independent proteinase activities (205). The localization of the hydrolase enzymes to the vacuoles indicates that they are lysosomal. The lysosomes have also been shown to take up ferritin (41), but the significance of this is unknown. Further investigation has shown that *G. lamblia* has two major cysteine proteases, of 40 and 105 kDa (145). The 40-kDa cysteine protease cleaves hemoglobin and immunoglobulin A1 (IgA1) (254) and has been proposed as a virulence determinant. Whether the proteases are involved in pathogenesis, life cycle switch, or another function is unknown.

Nuclei

The two nuclei of *Giardia* trophozoites are symmetrically placed on either side of the midline. The nuclear membrane is partially covered with ribosomes, and no nucleoli have been seen. Studies of nuclear replication by using [^{3}H]thymidine (328) and 4′,6-diamidino-2-phenylindole (DAPI) fluorescent staining (177) have suggested that both nuclei replicate at the same or nearly the same time. When DNA was stained with DAPI, approximately 1 of 100 cells had condensed structures, and when these structures were present, they were seen in both nuclei. Trophozoites have been metabolically labeled with [^{3}H]uridine (177), and the label was incorporated equally in both nuclei, indicating that both nuclei are transcriptionally active. Certain other protozoans, including the ciliates and *Dientamoeba fragilis*, also contain two nuclei. However, in the ciliates, a transcriptionally active macronucleus consisting of multiple copies of transcribed genes is formed from the micronucleus, which contains the entire genome in a single copy (330). *D. fragilis* trophozoites usually have two equal-appearing nuclei, although some trophozoites are mononuclear and a few organisms contain more than two nuclei. Cytokinesis of binucleate trophozoites results in two mononuclear organisms, followed by nuclear division and the reappearance of binucleate organisms (325). Thus, *Giardia* spp. are unique in possessing two nuclei that are equivalent by all criteria that have been applied. This raises the interesting question of why *Giardia* spp. have two nuclei and the selective advantage that maintains two nuclei. If each nucleus indeed has the same complement of chromosomes, is there any exchange of DNA between the two nuclei?

Endosymbionts

Ultrastructural studies have identified a variety of endosymbionts in *Giardia* trophozoites (108, 245). An endosymbiont morphologically consistent with a gram-negative bacterium was identified in a *G. muris* isolate that was especially infective for mice, but was not found in several *G. lamblia* isolates (245). In a subsequent study, bacteria and mycoplasmalike structures were seen in *Giardia* cysts and trophozoites from a variety of mammals and birds and viruslike particles were seen in *G. muris* trophozoites (108). One of the more interesting findings is the presence of a 33-nm double-stranded RNA virus (*G. lamblia* virus [GLV]) in certain human isolates of *G. lamblia* (316). It consists of a 7-kb double-stranded RNA genome surrounded by a 33-nm icosahedral capsule (223) and most probably replicates by means of a virus-encoded RNA polymerase (327). GLV can transfect uninfected isolates (75, 222) and is associated with decreased adherence and growth rate (223). The presence of an RNA virus in *Trichomonas vaginalis* has been correlated with the ability to undergo phenotypic variation (315), but GLV has not been associated with the ability of *G. lamblia* to undergo antigenic variation (2). A single-stranded copy of GLV RNA has been isolated (119) and has been introduced into previously uninfected *G. lamblia* trophozoites by electroporation; this has resulted in the production of complete viral particles (118).

CYST STRUCTURE

The cyst is the infective form of *Giardia* spp. and is relatively resistant to the external environment as well as to gastric acid in the stomach of the infected host. The cyst is approximately 5 by 8 μm and is surrounded by a wall that is 0.3 μm thick (286). The outer portion of the wall consists of a structure composed of 7 to 20 filaments (92) (Fig. 2), whereas the inner (membranous) portion contains an outer and inner cyst wall membrane separated by a thin layer of cytoplasm.

Galactosamine was detected as the major sugar of the outer cyst wall of in vivo-derived cysts by gas chromatography, mass spectrometry, and enzymatic assays (purified chitinase) (171). Binding to a lectin from *Phaseolus limensis* with an affinity for *N*-acetylgalactosamine (GalNAc) suggested that the galactosamine was present in the form of GalNAc. Glucose is the major sugar in the remainder of the cyst. Amyloglycosidase treatment of the outer cyst wall decreases the concentration of glucose 30-fold and abolishes periodic acid-Schiff staining, suggesting that the glucose is present as glycogen.

The presence of chitin in the cyst wall has been suggested on the basis of binding of the outer cyst wall to wheat germ agglutinin (WGA); this binding was abolished by chitinase (321). WGA binds to *N*-acetylglucosamine (GlcNAc), the sugar that composes chitin. The existence of chitin synthetase activity in encysting trophozoites (129) has also been proposed. However, these findings have been criticized because of the potential lack of specificity of the chemicals (171). In later studies, GlcNAc was detected by mass spectrometry of trophozoites and in vitro-derived cysts (247), but the purification process probably removed the insoluble outer cyst wall. The lack of staining of the cyst wall by

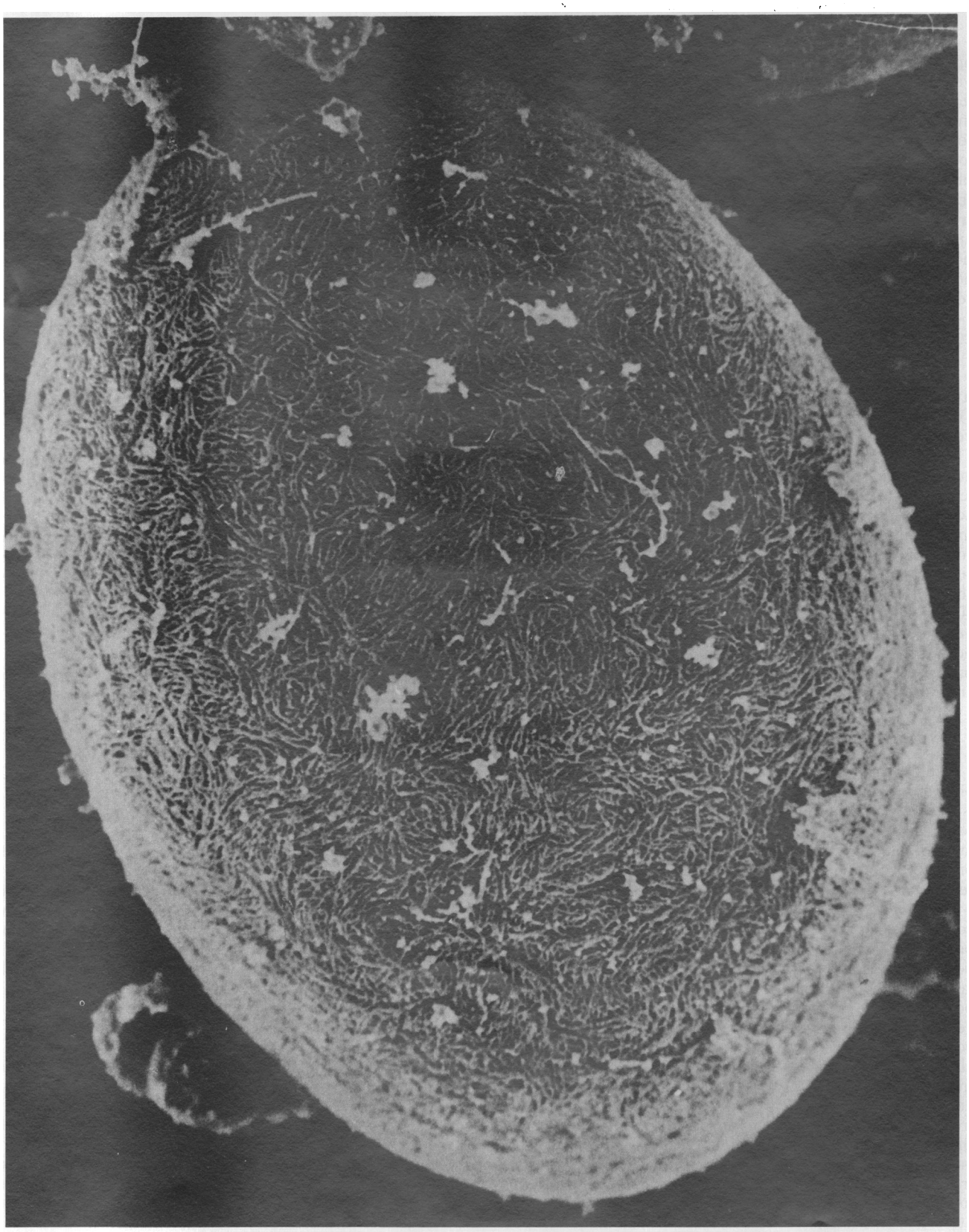


FIG. 2. High-resolution field emission scanning electron micrograph of a *G. muris* cyst, demonstrating the filamentous structure of the cyst wall. Courtesy of Stanley Erlandsen.

Zander's iodine-zinc chloride, Kuhnelt's sulfuric acid-iodine, and picro-nigroxine (168) and the absence of Feulgen staining after hydrolysis (114) also argue against the presence of chitin as a major component of the cyst wall.

The discovery of GalNAc in cysts but not in trophozoites (171, 247) may be explained by the observation that encysting *Giardia* trophozoites synthesize GalNAc from glucose through an inducible pathway (168). The activity of one enzyme of this pathway, UDP-GlcNAc 4′-epimerase, becomes detectable after 8 h in encystation medium and peaks at 48 h. Thus, synthesis of the outer cyst wall sugar, GalNAc, during encystation may be a key biochemical process of encystation.

The presence of protein in the cyst wall was demonstrated by the hybridization of cyst wall-specific antibodies to specific bands on Western immunoblots (266). Immunogold electron microscopy has been used to localize several proteins (29, 75, 88, and 102 kDa) to the filamentous structures of the outer portion of the cyst wall (93).

Inside the *G. lamblia* cyst wall, flagellar axonemes, vacuoles, ribosomes, and fragments of the ventral disk are found (92, 96, 286). Most cysts contain four nuclei, whereas those that have not yet undergone nuclear division contain two.

EXCYSTATION AND ENCYSTATION

Excystation of *G. lamblia* in vitro can be induced by imitating the acidic environment of the stomach. Studies have determined the optimum pH to be 1.3 to 2.7 (39) or 4.0 (45). However, excystation of *G. lamblia* (45) and *G. muris* (111) can also occur at a neutral pH, providing an explanation of how giardiasis occurs in patients with achlorhydria. Excystation of *G. muris* can be induced by 0.3 M sodium bicarbonate at pH 7.5 (111), suggesting that pancreatic secretions play a role in excystation.

The sequence of events that occur during the process of excystation has been determined by observing the in vitro process for *G. muris* (62, 63) and *G. lamblia* (49). Within 5 to 10 min after being placed in conditions conducive to excystation, flagellar motion begins and the posterior end of the trophozoite exits through a break in the cyst wall. Cytokinesis begins within 30 min, resulting in two binucleate trophozoites. During excystation, the vacuoles release their contents, leading to the speculation that enzymes from the vacuoles play a role (63). Small, dense vesicles are seen on the surface of the trophozoites as they emerge from the cyst wall.

More recently, encystation has also been performed in vitro (129, 284, 298), allowing full completion of the *G. lamblia* life cycle in vitro. Because maximal encystation of *G. lamblia* was observed in the mid to lower jejunum of infected suckling mice, these conditions were imitated in vitro (129). Optimal encystation was observed in the presence of the primary bile salts, glycocholate with myristic acid, at a pH of 7.8 (128). In other studies, porcine bile and lactate were effective in inducing encystation (122). Within the first 5 h of encystation, encystation-specific antigens can be found in a Golgi apparatus and appear to be packaged into encystation-specific vesicles, which appear in 6 to 18 h (105) and are transported to the forming cyst wall (267). Initially, a group of low-molecular-mass antigens (21 to 39 kDa) appear. At 24 h, four higher-molecular-mass antigens (66 to 103 kDa) appear, all of which react with WGA. It is likely that some of these antigens are the same as those that have been localized to the filaments of the cyst wall (93). By approximately 44 to 70 h, formation of the cyst wall is completed (267) and both nuclei divide simultaneously, yielding a cyst with four nuclei, whereas cytokinesis occurs shortly after excystation, resulting in the development of two trophozoites from one cyst. At least some of the in vitro-derived cysts can be induced to excyst into trophozoites. Triggering of excystation is maximal at pH 4.0, and emergence is stimulated by chymotrypsin, trypsin and pancreatic fluid (45).

BIOCHEMISTRY

Axenic Culture

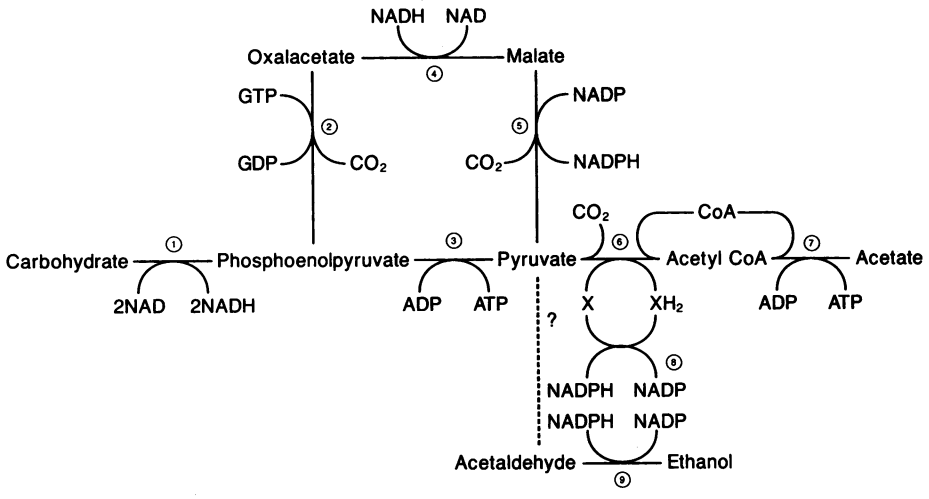
The development of an axenic (free of exogenous cells) medium for the growth of *G. lamblia* trophozoites in 1976 (220) has permitted studies of the biochemistry and nutritional requirements of *G. lamblia*. The first *Giardia* isolate axenized was from a patient in Portland, Oreg., and is called Portland-1 (P-1). Most in vitro studies of *G. lamblia* have used the P-1 or the WB isolate, which was axenized from a patient with prolonged symptomatic giardiasis who probably acquired the infection in Afghanistan (291). Although a totally defined medium has not been developed, certain observations can be made about the nutritional requirements of *Giardia* spp. The most commonly used medium, modified TYI-S-33 (183), includes bovine serum, bile salts, casein digest, yeast extract, cysteine, dextrose, ferric ammonium citrate, and ascorbic acid. Especially notable are the absolute requirements for cysteine and a low O_2 concentration, discussed in greater detail below.

Carbohydrate and Protein Metabolism

Although eukaryotes are characterized by a predominantly aerobic metabolism, several protozoans, including *Giardia*, *Entamoeba*, and *Trichomonas* spp., are notable exceptions (229). In keeping with their lack of mitochondria and mitochondrial enzymes, these organisms produce energy by fermentation, with a lack of complete oxidation of carbohydrates to CO_2 and H_2O. All the enzymes of carbohydrate metabolism in *Giardia* spp. are present in the cytoplasm, whereas some of the enzymes in *Trichomonas* spp. are present in organelles called hydrogenosomes (205). Simple sugars, primarily glucose, appear to provide the major source of energy that is derived from carbohydrates (206). In one study, *Giardia* trophozoites were able to proliferate in medium with a low (<10 mM) glucose concentration at a rate 50% that of controls, leading to the suggestion that *G. lamblia* trophozoites obtain a portion of their energy from sources other than glucose (283). However, the use of endogenous glucose or other energy stores was not ruled out.

Analyses of the carbohydrate metabolism of *G. lamblia* have demonstrated the absence of a Krebs cycle, cytochrome-mediated electron transport, and oxidative phosphorylation (206, 324). Cytochromes have not been detected in *Giardia* spp. (172).

Glucose and perhaps other simple sugars are converted to pyruvate by the Embden-Meyerhof-Parnas and hexose monophosphate shunt pathways (172, 206, 324) (Fig. 3). In most aerobic and anaerobic organisms, the major regulatory step in glycolysis is the conversion of fructose 6-phosphate to fructose 1,6-phosphate by ATP-dependent phosphofructokinase. However, like the anaerobic protozoans *Entamoeba histolytica* and *Trichomonas* sp., *G. lamblia* contains PP_i-dependent phosphofructokinase instead of ATP-



① Glycolytic enzymes; ② Phosphoenolpyruvate carboxykinase (GDP); ③ pyruvate kinase (ADP); ④ malate dehydrogenase (NAD); ⑤ malate dehydrogenase (decarboxylating)(NADP); ⑥ pyruvate: ferredoxin oxidoreductase ⑦ acetyl-CoA synthetase (ADP); ⑧ alcohol dehydrogenase (NADP); ⑨ NADPH oxidoreductase.

FIG. 3. Carbohydrate pathway. Note that the old name for no. 6 is pyruvate synthase. See reference 252 for inclusion of the alanine synthesis pathway. Reprinted with permission (206).

dependent phosphofructokinase (219). No evidence for regulation of this enzyme has been reported.

The end products of carbohydrate metabolism vary considerably with the O_2 concentration (252, 253). In an anaerobic environment, alanine is the major product (89, 252, 253). It has been proposed that alanine is produced from pyruvate and ketoglutarate in a pathway using glutamate dehydrogenase and alanine aminotransferase and the oxidation of NAD(P)H (252). With the addition of low concentrations of O_2 (<0.25 μM), ethanol production is stimulated and alanine production is decreased (252). At somewhat higher O_2 concentrations (>46 μM), acetate and CO_2 production is predominant and alanine production disappears (89). The oxygen-sensitive enzyme pyruvate:ferredoxin oxidoreductase (formerly called pyruvate synthase) catalyzes the production of acetyl coenzyme A from pyruvate, but whether this explains the inhibition of acetate production by high O_2 concentrations has not been reported.

In addition to alanine production, there is evidence for endogenous production of valine (252). The proposal that proline is synthesized (89) has not been confirmed (252). Therefore, most amino acids are obtained by scavenging from the host.

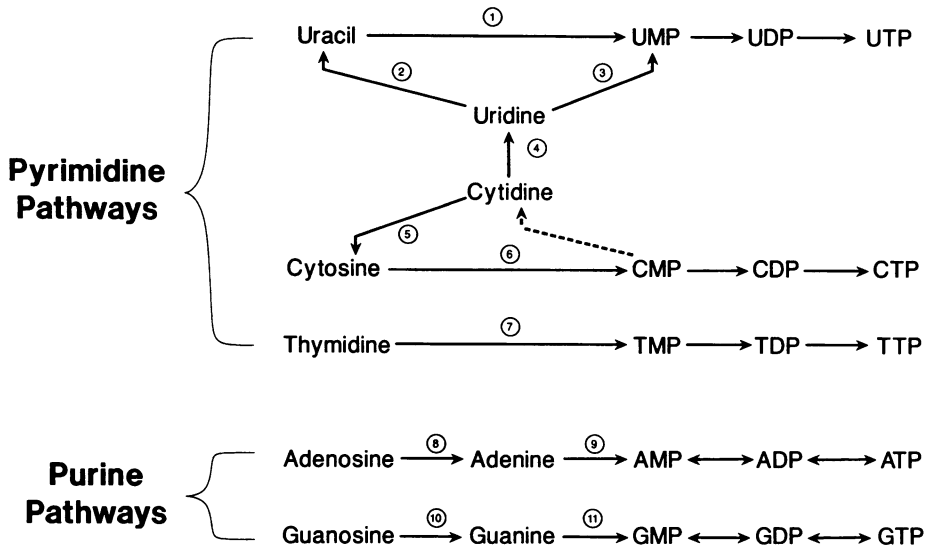
During axenic growth, *G. lamblia* trophozoites survive best under conditions of low O_2 tension. Survival in high O_2 (ambient concentration) is reduced, but cysteine provides partial protection against the O_2-mediated killing. This protection is a specific effect of cysteine and is not seen with cystine or other reducing agents (123, 124, 127). The presence of free thiol groups on the surface of *Giardia* trophozoites has been demonstrated by showing that trophozoites were killed by three different thiol-blocking agents that do not penetrate intact cells (130). The killing was prevented by cysteine and by proteins containing cysteine. When trophozoites are metabolically labeled with radioactive cysteine, most of the label is incorporated into a major surface protein that is present on the surface of the organism and secreted in large quantities into the medium (2, 8). Therefore, it is possible that this protein provides some protection against O_2.

Energy metabolism has been observed in *G. muris* cysts at a level approximately 10 to 20% that in trophozoites (251). Ethanol stimulated the respiration of both trophozoites and cysts, whereas glucose stimulated only trophozoites. The rate of respiration declined with decreasing temperature, suggesting a possible explanation for the longer survival of *Giardia* cysts at lower temperatures (38). Although most respiratory inhibitors had similar effects on cysts and trophozoites, metronidazole had no effect on cysts when it was used at concentrations that completely inhibited trophozoite respiration. Sodium nitrite is thought to act by a mechanism similar to that of metronidazole, and it inhibited respiration in both forms (251). Therefore it is proposed that metronidazole was not active against the cyst because it did not penetrate the cyst wall. Menadione, a redox-cycling naphthoquinone, first stimulated and then inhibited respiration in both cysts and trophozoites and destroyed their viability, presumably through the generation of O_2 radicals.

Lipid Metabolism

Studies of the lipid metabolism of *G. lamblia* trophozoites have revealed little or no de novo synthesis of cellular phospholipids or sterols (172). Rather, fatty acids and cholesterol are incorporated from the growth medium into the lipid fraction of the trophozoite. Although it is not known how much of the lipid synthesis is from free fatty acids and how much is from esterified fatty acids, trophozoites have been shown to incorporate arachidonic acid into phosphatidylinositol (40).

The importance of bile in the growth of *Giardia* spp. was first suggested by the propensity of the organisms to colonize the duodenum and jejunum. The enhanced growth of trophozoites in the presence of biliary lipids and the ability of biliary lipids to support their growth in the absence of serum suggest that bile may be important in vivo as a source of lipids (101, 104, 125). Specifically, in vitro growth of trophozoites is supported by cholesterol, the biliary phospholipid phosphatidylcholine, and the bile salts glycocholate and glycodeoxycholate (125). In contrast, free fatty acids at



① uracil phosphoribosyltransferase; ② uridine hydrolase; ③ uridine phosphotransferase; ④ cytidine deaminase; ⑤ cytidine hydrolase; ⑥ cytosine phosphoribosyltransferase; ⑦ thymidine phosphotransferase; ⑧ adenosine hydrolase; ⑨ adenine phosphoribosyltransferase; ⑩ guanosine hydrolase; ⑪ guanine phosphoribosyltransferase.

FIG. 4. Nucleotide synthesis pathway. Note that other investigators (196) have reported a uridine-thymidine phosphorylase catalyzing the conversion of uridine to uracil (enzyme no. 2) and thymidine to thymine, but the results have not been confirmed. Adapted with permission (13, 318).

concentrations below 12 μM are toxic to trophozoites. Normal human milk is toxic to trophozoites in vitro, probably through free fatty acids released by a bile salt-stimulated lipase present in the milk (268).

Nucleic Acid Salvage

Most eukaryotes are capable of de novo purine and pyrimidine synthesis, but notable exceptions are found among the protozoans (317). Although most of the protozoan parasites lack purine synthesis, *G. lamblia* (13) (Fig. 4), *Trichomonas vaginalis* (319), and *Tritrichomonas foetus* (320) also lack de novo synthesis of pyrimidines; instead, they depend on salvage of exogenously synthesized nucleic acids. Studies of the purine metabolism of *G. lamblia* by using radiolabeled precursors revealed the incorporation of adenine, adenosine, guanine, and guanosine into nucleotides, but not formate, glycine, hypoxanthine, inosine, or xanthine (35, 221, 318). Assays for enzyme activity revealed only adenosine and guanosine hydrolase and adenine and guanine phosphoribosyltransferase. The latter has been purified (14). *Giardia* spp. lack ribonucleotide reductase and fail to incorporate purine bases and nucleosides, instead depending on the salvage of exogenous purine deoxynucleosides (24).

Studies of pyrimidine metabolism have revealed dependence on salvage of exogenous thymidine, cytidine, and uridine (13, 204) (Fig. 4). The pyrimidines are taken up by active-transport mechanisms, and competition experiments suggest that one site is used for uridine and cytosine and another is used for thymidine (170). Other investigators have suggested that conversion of the pyrimidine nucleosides, thymidine, uridine, and deoxyuridine, to their respective bases may be catalyzed by a single enzyme, uridine/thymidine phosphorylase (196). These investigators did not find evidence for the enzyme uridine hydrolase (Fig. 4, enzyme no. 2) described by other investigators (13).

GENETICS

Analysis of *Giardia* by classic genetic analysis would be very helpful in understanding its biology. However, the lack of a defined growth medium with nutritional auxotrophs or drug-resistant mutants has made the classic approach impossible. Stable transformation with foreign DNA has also been difficult to achieve in protozoans, but transient (28, 60, 194) or stable (182, 193) expression of extrachromosomal DNA introduced by electroporation and stable expression with the use of genes integrated into the genome by homologous recombination (197) have recently been reported for other protozoans and may also be successful in *Giardia* spp. Thus far, our understanding of *Giardia* genetics has been obtained by analysis of the few *Giardia* genes that have been cloned.

DNA and Chromosome Content

The G+C content of the *G. lamblia* genome has been calculated to be 42% (242) or 48% (42), whereas the G+C content of the rDNA gene is 75% (146) and those of the protein-coding genes sequenced so far range from 49% to greater than 60% (Table 1). A satellite band containing rDNA can be seen on cesium chloride-ethidium bromide ultracentrifuged DNA preparations (1) because of the high G+C content. Therefore the noncoding regions must be relatively A+T rich if the values obtained by using the melting temperature are correct.

Estimates of the genome complexity of *G. lamblia* using C_0t analysis have ranged from 3.0×10^7 (242) to 8×10^7 (42).

TABLE 1. Protein-coding genes of *G. lamblia*

Protein[a] (reference)	Sequence at probable site of translation initiation[b]	Length of 5′ untranslated sequence[c]	3′ Untranslated sequence[d]	G+C content of protein-coding portion (%)
Structural proteins				
α-Tubulin (g, c) (187)	ATAAAAATGCGT	6		
β-Tubulin (g, c) (186, 187)	TTAAAAATGCGT	6	TAGCCTTGAGCGAGTAAATTCCCG	67
α_1-Giardin (g) (15, 256)	TTAAAAATGCCG	3	TAGGGGTTTAGTGAACGTCTTTAG	60
α_2-Giardin (15)	TAGAAAATGCCG	3	TAGGCGCCTTTACTGCGGGGTTTCCTTTCGCTAGTGA ATTGCCTAGCGGGT	57
β-Giardin (g, c) (6, 22, 162)	CCGTCTATGTTC		TAACGCCTCGAGTAAA	59
γ-Giardin (246)	AAGAAAATGAAG		TAAGGGGCTGGGCTGGTGAGTAAATTTCCCTATAGC	53
Surface proteins				
CRP 170 (g, c) (2, 5)	GCCTCAATGTTG	1	TGACTTAGGTAGTGAATGCTGTAC(A)$_n$	65, 56[e]
TSA 417 (g) (126)	GGCCTAATGTTC		TAGATGTACTTAGATAGTAAACCGTCATCGATG	53
CRP 72 (c) (5)			TGACTTAGGTAGTGAATGCTATAC(A)$_n$	55
VSP 1267 (c, g) (228)	ACTTCAATGTTG	1	TAGACTTAGGTAGTAAACGCGTCACTGT(A)$_n$	49
Heat shock protein (g) (7)	AGTAGGATGCCT			50
Glutamate dehydrogenase (g, c) (331)	TTTAAAATGCCT	3–6	TGAGGGCTGAAGTGAATATTTACCTTTTCC	61

[a] g, genomic; c, cDNA.
[b] In each case, the first ATG was used as the site of translation initiation and was in the open reading frame. For α_1-giardin, confirmation of the beginning of translation was obtained by N-terminal sequencing of the protein (15, 256). For the tubulin protein genes, comparison with tubulin genes from other organisms was consistent with the putative translation initiation site.
[c] Length in nucleotides. Determined by primer extension and/or S1 nuclease protection.
[d] The stop codon and possible polyadenylation signal (AGTPuAAPy) are underlined.
[e] The G+C contents of the repeat and 3′ regions of the CRP 170 gene are separated.

The number of chromosomes per nucleus has been estimated to be four by light microscopy (114) and by light-microscopic observation of fluorescent DAPI-stained nuclei (177).

In contrast, pulsed-field separations of intact chromosomes demonstrated the presence of at least five distinct sets of chromosomes in addition to the frequent occurrence of more faintly staining minor bands that showed marked DNA similarity to a major band of a slightly different size (3) (Fig. 5). The chromosome sizes varied between approximately 1×10^6 and 4×10^6 bp, yielding a total of 1.2×10^7 bp for the five chromosomes. Similarly, other investigators have estimated the haploid genome size to be approximately 1.1×10^7 bp by densitometric scanning of restriction digests of *G. lamblia* DNA with an infrequently digesting enzyme (99). Densitometric scanning suggested that each trophozoite contains approximately 30 to 50 chromosomal DNA molecules (3). This represents approximately one copy of the minor bands and three to five copies of the major bands per nucleus. One possible explanation of the data is that *Giardia* trophozoites are polyploid, containing several copies of each chromosome per nucleus. The minor bands might represent size variants of "homologous" chromosomes. Alternatively, each of the more intensely staining bands may represent more than one distinct chromosome.

The light-microscopic findings are most consistent with the presence of haploid nuclei. However, four (if each nucleus has the same chromosomes) or eight (if each nucleus has different chromosomes) chromosomes of 1 to 4 Mb do not explain the C_0t analysis values of 3×10^7 and 8×10^7 or the apparent presence of 30 to 50 chromosomal DNA molecules per trophozoite. Further studies involving different approaches are necessary to resolve these conflicting data regarding the *Giardia* genome size and ploidy.

If *Giardia* is polyploid, how are multiple copies of a chromosome maintained in the absence of a sexual cycle? To date, a sexual cycle of reproduction has not been described for *Giardia* spp. In addition, the remarkable genetic diversity among *Giardia* isolates can perhaps be explained better on the basis of clonal divergence (304) than through sexual reproduction.

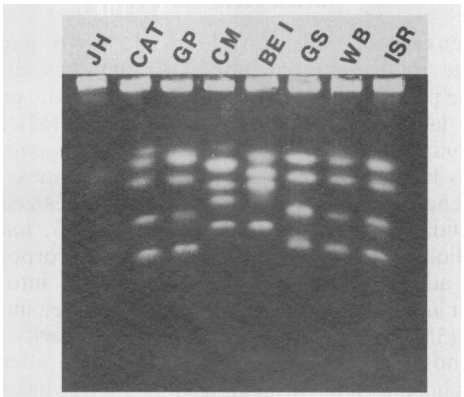


FIG. 5. Pulsed-field gel electrophoresis of eight isolates. Chromosomal separations of eight different *Giardia* isolates is shown. All are human except BE 1 (beaver) and GP (guinea pig). The JH isolate has five distinct chromosomes, and total DNA and chromosome-specific probes from the smallest two JH chromosomes hybridize to the smallest band from the other isolates, suggesting that it represents two comigrating chromosomes (3). Note the more faintly staining minor bands on some isolates (e.g., ISR). They are present in doubly cloned isolates and show marked DNA similarity to one of the more intensely staining bands, but the exact relationship between them is not known. Also note the distinct pattern of the CM and BE 1 isolates. In contrast, BE 2 migrates in a pattern similar to that of WB (1). It is of interest that the differences are distinct from the mammalian species of origin.

rRNA

The rRNAs of *G. lamblia* are unique in that they are smaller than those of other eukaryotes and in fact are smaller than those of the eubacteria (42, 83). The rDNA gene is only 5,566 bp (146) and is tandemly repeated in the genome. The tandem repeat encodes the large subunit (28S), small subunit (16S), and 5.8S forms, each of which is smaller than its counterparts in other eukaryotic organisms. In contrast, the rDNA genes of *G. muris* and *G. ardeae* are 7.6 kb because of a larger intergenic spacer region (311) (see the section on species determination and strain variation).

Sequence analysis of the gene encoding the small-subunit RNA has been used to classify *G. lamblia* as the most primitive eukaryote (296). In fact, the sequence of the *G. lamblia* small-subunit rRNA shows greater similarity to the archaebacteria sequence than do the sequences from other eukaryotes. The small size has also allowed further analysis of the essential regions and putative processing regions (85).

Studies of three *G. lamblia* telomeric clones revealed an abrupt transition from rDNA to telomeric repeats, TAGGG, two from the same place at the beginning of the large-subunit rRNA and one at the beginning of the intergenic spacer. The sequence CCCCGGA was present at each of the breakpoints (4). Copies of rDNA genes were present on multiple chromosomes, with substantial variation among otherwise closely related isolates. Interestingly, copies of the rDNA repeats are present on the minor bands of the ISR isolate and may account for some of the size difference of the minor bands. Other investigators also demonstrated a telomeric location for rDNA repeats and showed frequent rearrangements of the rDNA repeats within cloned lines of WB trophozoites (195a).

Translation

Little is known about translation in *Giardia* spp., but there is some evidence to suggest that translation initiation may be different from that in the higher eukaryotes. Of the sequences that have been reported from cDNA clones and confirmed by RNA sequencing of the 5′ end and/or S1 mapping, a very short 5′ untranslated region has been discovered (1 to 6 bases [Table 1]). In contrast, only 4 of 699 vertebrate mRNAs had leader sequences less than 10 nucleotides in length (190). In vertebrates, mRNAs with short leader sequences are generally translated inefficiently.

The mechanism proposed for eukaryotic translation initiation is the scanning model (191). The 5′ nucleotide of mRNA contains a methylated cap structure which stabilizes the mRNA and is involved in formation of the secondary and in splicing. In the scanning model, the small-subunit RNA binds the cap structure and moves along (scans) the mRNA until it reaches the first AUG, where translation is initiated. (Although the first AUG is used for translation initiation in most mRNAs, a later AUG is occasionally used.) The short leader sequences found in *G. lamblia* mRNAs suggest that in *G. lamblia*, binding of the ribosome to the mRNA and initiation of translation may be more closely linked than in vertebrates. Relatively short leader sequences are also found in the actin (11 bases [86]) and ferredoxin (9 bases [164]) genes of *Entamoeba histolytica* and the ferredoxin gene (16 bases [173]) of *Trichomonas vaginalis*. The kinetoplastic protozoans, including *Trypanosoma* sp. and *Leishmania* sp., contain *trans*-spliced leader RNA sequences which are 39 nucleotides long in the mature mRNA (78, 314), but no evidence has been found for a similar phenomenon in *Giardia* spp.

The eukaryotic ribosome recognition sequences include the initiation codon, and consensus sequences are GCCA/GCCAUGG in vertebrates (190), C/AAAA/CAUG in *Drosophila melanogaster* (56), and A/YAA/UAAUGUAU in *Saccharomyces cerevisiae* (58). There is little similarity of the region surrounding the initiation among the *G. lamblia* sequences or with the vertebrate consensus sequence. However, the α- and β-tubulin sequences match the *Drosophila* and *S. cerevisiae* recognition consensus sequences, and A is present in 7 of 11 genes at the −3 position and in 9 of 11 at the −1 position. It must be emphasized that for the *Giardia* sequences reported, the correct initiation codon has not been experimentally determined by N-terminal amino acid sequencing, with the exception of α_1-giardin (15, 256); knowledge of this is essential for more rigorous analysis of ribosome recognition sequences in *Giardia* spp. Despite the differences between mRNA from *Giardia* spp. and vertebrates, in vitro translation of *Giardia* mRNA has been performed in a rabbit reticulocyte lysate system (11, 256), indicating that *G. lamblia* mRNA can be translated in a vertebrate system. In addition, a comparison of the peptidyltransferase loop sequence from domain V of the *G. lamblia* LS rRNA reveals greater similarity to eukaryotes than to archaebacteria or eubacteria (82).

The prokaryotic ribosome recognition (Shine-Dalgarno) sequence (consensus UAAGGAGG) is located 5 to 10 nucleotides before the initiation codon. A Shine-Dalgarno sequence has been proposed in some of the *Giardia* sequences analyzed, but there has been no consistent relationship between the location of the putative Shine-Dalgarno sequence and the initiation codon. In addition, the short 5′ leader sequences would not allow ribosomes to recognize an upstream sequence. Thus, although the translation apparatus of *G. lamblia* is significantly different from that of other eukaryotes, it appears to be more similar to that of other eukaryotes than of prokaryotes.

Studies of translation initiation in *Giardia* spp. promise to be interesting and may shed light on the relationships between *Giardia* spp., the prokaryotes, and the higher eukaryotes. The short 5′ leader sequences and the unique rRNA may even allow insight into the truly essential features of eukaryotic translation.

Transcription

Transcription may be more similar to that in other eukaryotes. A possible TATAA box has been identified in the genomic clones that have been sequenced (7, 126, 187) 9 to 134 bases upstream from the initiation codon. Whether any of these potential TATAA sequences represent sites recognized by RNA polymerase is unknown. A poly(A) tail follows a short 3′ untranslated region (Table 1). A 6- to 19-nucleotide interval separates the sequence AGTPuAAPy from the stop codon, which is then separated by 7 to 10 bases from the beginning of the poly(A) tail. The sequence AGT PuAA has been proposed as a polyadenylation signal for *Giardia* spp. (256), and, in addition, a pyrimidine is present at the next base in each of the reported sequences. In comparison, the polyadenylation consensus sequence in vertebrates and plants (AATAAA) is found 10 to 30 nucleotides from the poly(A) tail (264).

Introns have been identified in only a minority of protein-coding genes of other protozoans, and, so far, no introns have been reported in *Giardia* spp.

Heat Shock Response

Analysis of the heat shock response in *Giardia* spp. revealed repression of normal protein synthesis and increased synthesis of four proteins (100, 83, 70, and 30 kDa) (203). In addition, the sequence of a gene whose transcript level increases during heat shock but is unrelated to the HSP70 genes of other organisms has been reported (7). This gene does not hybridize to DNA from certain isolates (e.g., the GS isolate [232]), providing further evidence that this gene does not encode a typical heat shock protein.

SPECIES DETERMINATION AND STRAIN VARIATION

Determination of *Giardia* species has classically been made by two predominant methods: (i) host of origin, and (ii) morphology by light microscopy of the trophozoite, primarily by the appearance of the median body. Since *Giardia* spp. colonize many mammalian and nonmammalian hosts, the classification scheme depending on the host of origin resulted in numerous *Giardia* species, each named for the host of origin (147). In contrast, three distinct morphologic types of trophozoites were identified, *G. lamblia* or *G. duodenalis* (mammals and birds), *G. muris* (mouse), and *G. agilis* (tadpole) (114). Species determination by host of origin is probably invalid because *Giardia* spp. from different hosts (e.g., cat, guinea pig, beaver) frequently appear identical and are very similar by DNA analysis, whereas different isolates from the same host species can be markedly different (242). Transmission from one host to another has been successful in a number of experiments (97, 188), although results have sometimes been contradictory. In contrast to the difficulties of species determination by host of origin, electron microscopy of the cyst and trophozoite has improved the ability to detect morphologic differences between organisms that appear the same by light microscopy.

The lack of a gold standard for species determination in protozoans (304) adds to the difficulty of assigning appropriate species to various *Giardia* isolates. The question of whether giardiasis is a zoonotic infection has been controversial (34, 106), and its resolution depends on clarifying the issue of species determination. Therefore, the differences among the *Giardia* species and isolates will be summarized below to help clarify the current state of knowledge.

Light-Microscopic Categories of *Giardia* Species

G. agilis is a parasite of amphibians and has not been isolated from mammals. The trophozoites are long (13 to 36 μm) and narrow (4 μm) (110), flex at the midbody, and have long club-shaped median bodies. In vitro growth has not been reported for *G. agilis*.

G. muris trophozoites are approximately 10 to 12 μm by 5 to 7 μm, have a small, rounded median body (117), and are found in rodents, reptiles, and birds. Like *G. agilis*, *G. muris* has not been grown in vitro and has not been shown to cause human infection. Mice infected with *G. muris* have provided a good animal model for studying the immune response to giardiasis (see below).

Trophozoites of the *G. lamblia* group are approximately 10 to 12 μm by 5 to 7 μm and have one or two median bodies that resemble claw hammers. Organisms of this form have been isolated from humans, dogs, cats, guinea pigs, beavers, gerbils, birds and reptiles, sheep, and cattle, and at least some of the animals are believed to act as a reservoir for human infection (50, 106). Trophozoites from this group have been axenized and have therefore been studied in greater detail than *G. agilis* and *G. muris*.

Electron-Microscopic Categories of the *G. lamblia* Group

Electron-microscopic studies of *Giardia* cysts isolated from voles demonstrated the presence of the organelles of two trophozoites within one cyst, suggesting that cytokinesis occurs within the cyst rather than after excystation as in other organisms from the *G. lamblia* group (107). Therefore, these organisms have been assigned to a distinct species, *G. microti*, on the basis of functional and morphologic criteria. The muskrat belongs to the same family as the vole (Cricetidae) and has a *Giardia* sp. of similar morphology; thus, the muskrat *Giardia* sp. may also belong to the species *G. microti*.

Similarly, a *Giardia* sp. isolated from a parakeet was classified in the *G. lamblia* group on the basis of the characteristic claw hammer-shaped median body. Investigation of the trophozoites by scanning electron microscopy demonstrated an incomplete ventrolateral flange and lack of a marginal groove; accordingly, these isolates have been assigned to a separate species, *G. psittaci* (91). A *Giardia* sp. isolated from the great blue heron is also morphologically distinct from other species and has been called *G. ardeae* (94). The median body is similar to those of *G. muris* and *G. lamblia*, but the ventral disk and single caudal flagellum are more similar to those of *G. muris*.

Nonmorphological Differences among the *Giardia* Species

Nonmorphological differences between the morphological *Giardia* groups have also been shown. Pulsed-field separations of the chromosomes of *G. lamblia*, *G. muris*, and *G. ardeae* demonstrated marked differences in the sizes and apparent number of chromosomes, and at very high stringency, little cross-hybridization of total DNA or specific DNA probes was observed among the three species (51). A comparison of the rDNA genes of *G. lamblia*, *G. muris*, and *G. ardeae* has revealed significant differences (311). The rDNA genes of *G. muris* and *G. ardeae* are significantly larger (7.6 kb) than those of *G. lamblia* (5.6 kb), primarily because of a much larger intergenic spacer region in *G. muris* and *G. ardeae*. Differences were also found in the coding regions for the 5.8S rRNA and the 3′ region of the LS rRNA (311).

Nonmorphological Differences among *G. lamblia* Isolates

Significant difference have also been demonstrated among isolates of the *G. lamblia* group by nonmorphological criteria. Despite the common occurrence of *G. lamblia* infection in dogs, well-documented cases of transmission from dogs to humans or from humans to dogs are rare (55). In one study, attempts at axenization were successful in 44% of human isolates and none of 24 dog isolates, suggesting a biological difference between *G. lamblia* isolates obtained from humans and dogs (216). In contrast, *G. lamblia* isolates obtained from cats are more easily axenized and are similar to human isolates (215, 242, 303). Like human isolates, cat isolates could be transmitted to gerbils, but not to mice (188); however, in another study, human *Giardia* isolates were not easily transmitted to cats (189).

Differences have also been found in isoenzyme patterns (37, 57, 215), DNA restriction patterns (242), chromosome patterns (3, 51, 76, 310) (Fig. 5), surface antigens (241), the

ability to axenize (216), virulence (10, 240), and drug susceptibility (43, 213). These studies have included isolates from humans, cats, beavers, and guinea pigs, and the above differences are not clearly associated with host of origin. For example, the GS and CM isolates are different from most other human isolates (e.g., WB, P-1) in the following ways: GS has a totally different set of variant surface antigens (232), and a clone of the CRP170 gene (2) does not hybridize to DNA from GS and a number of other isolates (236). In addition, a cloned gene of a heat shock-inducible protein (7) does not hybridize to DNA from these isolates (232), and chromosome patterns from these isolates are different when studied by pulsed-field electrophoresis (3). Interestingly, of two axenized beaver isolates, one (Be-2) is similar to WB while the other (Be-1) is different from both WB and GS (236). A study of the karyotypes of 54 *G. lamblia* isolates revealed that the karyotype showed a greater correlation with the geographic origin of the isolate than with the host of origin (310), a finding that is consistent with the thesis that *G. lamblia* is not restricted to a single host.

Assessment of the relevance of some of these criteria is further complicated by the variation in chromosome size (3), surface antigen (2, 234), and drug susceptibility (43, 44) that occur within single isolates.

The substantial differences at the DNA level (e.g., different repertoires of surface antigen genes) suggest that there may be more than one human species (or subspecies) within the *G. lamblia* group. In summary, it appears likely that giardiasis can be a zoonosis, but this is not proven and the extent of zoonotic (versus human-to-human) transmission is unknown. Future investigations of human outbreaks of giardiasis potentially resulting from an animal reservoir would be greatly aided by axenization of isolates from humans and animals. The isolates could then be compared for ultrastructural and DNA relatedness. The marked variation of the rDNA sequences among species and the similarity of the rDNA sequences within the *G. lamblia* isolates that have been examined suggest the potential usefulness of rDNA sequences in determining the source of infection in outbreaks.

EPIDEMIOLOGY

G. lamblia is the most commonly isolated intestinal parasite throughout the world and is especially prevalent in children in developing countries (102, 134, 210, 211). Children in rural Guatemala were all infected during the first 3 years of life (211). Seroprevalence studies demonstrated evidence of past or present infection in 40% of Peruvian children by the age of 6 months (224), and examinations of stools from children in Bangladesh and Zimbabwe showed prevalence rates of approximately 20% (134, 210). Reinfection after spontaneous (134, 211) or induced (135) cure is very common. Although the majority of these infections are asymptomatic, some children have chronic diarrhea and weight loss (102, 211).

The *Giardia* cyst is highly infectious for humans, and patent infections can be established by the ingestion of as few as 10 viable cysts (269). The cyst can be ingested from a variety of different sources. *G. lamblia* is the most common cause of epidemic waterborne diarrheal disease, and numerous outbreaks of giardiasis associated with contaminated water have been reported. Investigations of waterborne outbreaks have implicated inadequate water treatment, simple chlorination only, contamination of the water main by sewage, or cross-connections to or back siphonage of sewage (65, 66). Infected animals such as beavers have also been associated with waterborne outbreaks (79). The occurrence of giardiasis in backpackers who drank water in regions with no human inhabitation (23) has also suggested that beavers or other wild animals are reservoirs. As noted above, confirmation of zoonotic transmission of *G. lamblia* to humans will require more rigorous comparison of human and animal isolates from individual outbreaks. *Giardia* cysts are moderately susceptible to killing by ozone and the halogens (169), but are not routinely susceptible to the usual levels of chlorine recommended for drinking water; therefore, outbreaks of giardiasis can occur from water that has an acceptably low level of coliform organisms (66). Sand filtration is considered to be the most effective treatment for removal of *Giardia* cysts (209). Waterborne transmission has also been implicated as a cause of giardiasis in travelers. Despite the high prevalence of giardiasis in developing nations of the tropics, giardiasis has more frequently been reported in travelers to northern countries. Travel to Leningrad is a notable cause of giardiasis, and more than 20% of travelers (in individual groups the number was as high as 90%) to Leningrad have acquired symptomatic giardiasis (47). Another study of travelers to Leningrad showed that 55% of those who drank tap water and 25% of those who did not became infected (175). Perhaps waterborne epidemics occur in cooler climates because *G. lamblia* cysts survive well at low temperatures (more than 2 months at 4°C and less than 4 days at 37°C) (38).

Direct fecal-oral transmission is the other major means by which *G. lamblia* is spread. The prevalence of giardiasis is increased in children attending day care centers and may be as high as 35% (259, 261, 329). Although the children usually have asymptomatic infections, transmission of their infections may result in symptomatic giardiasis in family members or others (261). The increased prevalence of giardiasis in homosexual men is most probably the result of fecal-oral transmission (282).

Food-borne transmission is a less common but well-documented source of giardiasis (248, 258, 326). These outbreaks have most probably occurred through contamination of freshly prepared food by an infected food handler.

CLINICAL ILLNESS AND PATHOGENESIS

The clinical manifestations of giardiasis vary from asymptomatic infection to chronic diarrhea with malabsorption. Studies of travelers to Leningrad have indicated that the incubation period (from infection until the development of symptoms) averages 1 (174) to 2 (47) weeks, but varies from 1 to 45 days (47). In human volunteers inoculated with *G. lamblia* trophozoites by intestinal intubation, the incubation period was 1 week (240). Patients with symptomatic giardiasis have diarrhea with loose, foul-smelling stools that are greasy, frothy, or bulky. Other common gastrointestinal symptoms include abdominal cramps and bloating, nausea, and decreased appetite. Malaise and weight loss are seen in the majority of patients, and fever is occasionally present, especially in the early part of the infection (47, 225). Villous shortening (Fig. 6) is variably present, and a mild chronic inflammatory response is seen during resolution of infection. Histologic examination may reveal trophozoites on the mucosal surface (Fig. 6 and 7) or possibly invading the mucosa or epithelial cells (163). In untreated patients, the median duration of illness is approximately 6 weeks, with symptoms seldom lasting less than 1 week (47, 225). The long duration of symptoms is in marked contrast to the illness caused by

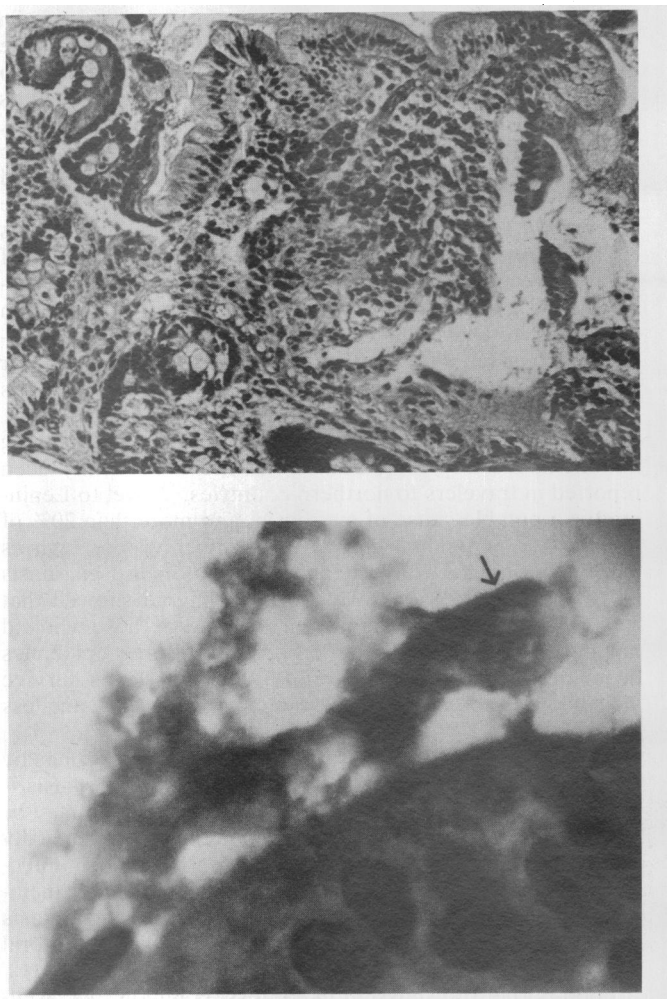


FIG. 6. (Top) A jejunal biopsy from a patient with giardiasis demonstrates flattening of the intestinal villi and a mononuclear inflammatory response. (Bottom) A high-power magnification from the same biopsy demonstrates a *G. lamblia* trophozoite (arrow) attached to the intestinal epithelium. Photographs courtesy of Richard Sobonya.

most viral and bacterial agents of diarrhea, and giardiasis should be considered in the differential diagnosis of patients with chronic diarrhea.

Patients with hypogammaglobulinemia are at increased risk of severe giardiasis characterized by chronic diarrhea and malabsorption (17, 18, 48, 148, 163, 208, 255, 257). Most have common variable hypogammaglobulinemia, but some have congenital hypogammaglobulinemia or occasionally selective IgA deficiency. Many have nodular hyperplasia of the small intestine. Whether selective IgA deficiency is a risk factor for giardiasis remains controversial. IgA deficiency is common in the general population (10% prevalence), suggesting that most people with IgA deficiency handle *Giardia* infections normally. Stool examinations are sometimes negative, but *G. lamblia* trophozoites can be found in the duodenal contents or by histologic examination of the duodenum and upper jejunum. Vitamin B_{12}, lactose, fat, and protein absorption is decreased. The pathologic changes are generally more marked than those in immunocompetent patients. The villi may vary from normal to markedly abnormal within a single patient, and lymphoid hyperplasia and bacterial overgrowth are common. After successful treatment of the giardiasis, the villus architecture returns to normal and the malabsorption resolves (17, 132). The lymphoid hyperplasia may (323) or may not (17) resolve with treatment.

Extraintestinal manifestations have rarely been reported in patients with giardiasis; when they do occur, they include urticaria (61, 100, 144), reactive arthritis (139, 285), and retinitis (20). It has been proposed that these complications are the result of the host immune response to infection.

Giardiasis is very common among children in developing countries (210, 287), as well as children in day care centers in developed countries (259), but in contrast to epidemic giardiasis, most of the infected children are asymptomatic. Some studies have shown no effect on weight or nutritional status (280, 287), whereas others have shown an association between giardiasis and lactose malabsorption and/or malnutrition (102, 305). The reason for the differences in clinical manifestations are not entirely clear but may include differences in host factors, as well as the pathogenicity of different *G. lamblia* isolates. In the epidemic at Aspen, Colo. (225), long-term residents had a much lower rate of infection than skiers visiting from elsewhere, and in a study of another epidemic (166), short-term (<2 years) residents had a much higher attack rate than long-term residents, suggesting that the incidence of symptomatic infection is decreased by prior exposure and subsequent immunity.

Studies of gerbils (10) and human volunteers (240) infected with two different *G. lamblia* isolates showed a marked difference in pathogenicity between the two isolates. All 10 human volunteers inoculated with one isolate (GS) were infected, and 50% developed symptoms, whereas none of 5 volunteers inoculated with the ISR isolate were infected. In subsequent studies, human volunteers were inoculated with two cloned GS isolates expressing different variant surface antigens (72 and 200 kDa) (239). All 4 inoculated with the GS clone expressing the 72-kDa antigen were infected, whereas only 1 of 13 inoculated with the clone expressing the 200-kDa antigen were infected. These observations indicate differences in virulence among isolates and among surface antigen variants from the same isolate. The correlation between resistance to trypsin and chymotrypsin and expression of different variant surface antigens (243) (see below) suggests a possible mechanism for the virulence differences among trophozoites expressing different surface antigens.

IgA proteases are found in many microbial intestinal pathogens and are probably important virulence determinants that allow them to colonize the intestinal mucosa despite a secretory immune response (185). The identification of IgA1 protease activity in *G. lamblia* trophozoites (254) suggests the possibility that *Giardia* spp. can survive in the intestine by degrading host IgA. However, most *Giardia* proteinases are bound to intracellular vesicles. Whether the molecule(s) with IgA protease activity is released from the organism and whether it degrades host IgA has not been reported.

The mechanism by which *G. lamblia* causes diarrhea is unknown. Superficial invasion of intestinal mucosa and epithelial cells has occasionally been found in infected humans (46, 226, 281) and in *G. lamblia*-infected mice (120) and has been correlated with the presence of steatorrhea (281), but the reports of intestinal invasion by *Giardia* spp. are not universally accepted (250). Bacterial overgrowth is common in patients with giardiasis and malabsorption and has been proposed as a possible cause of the diarrhea (300,

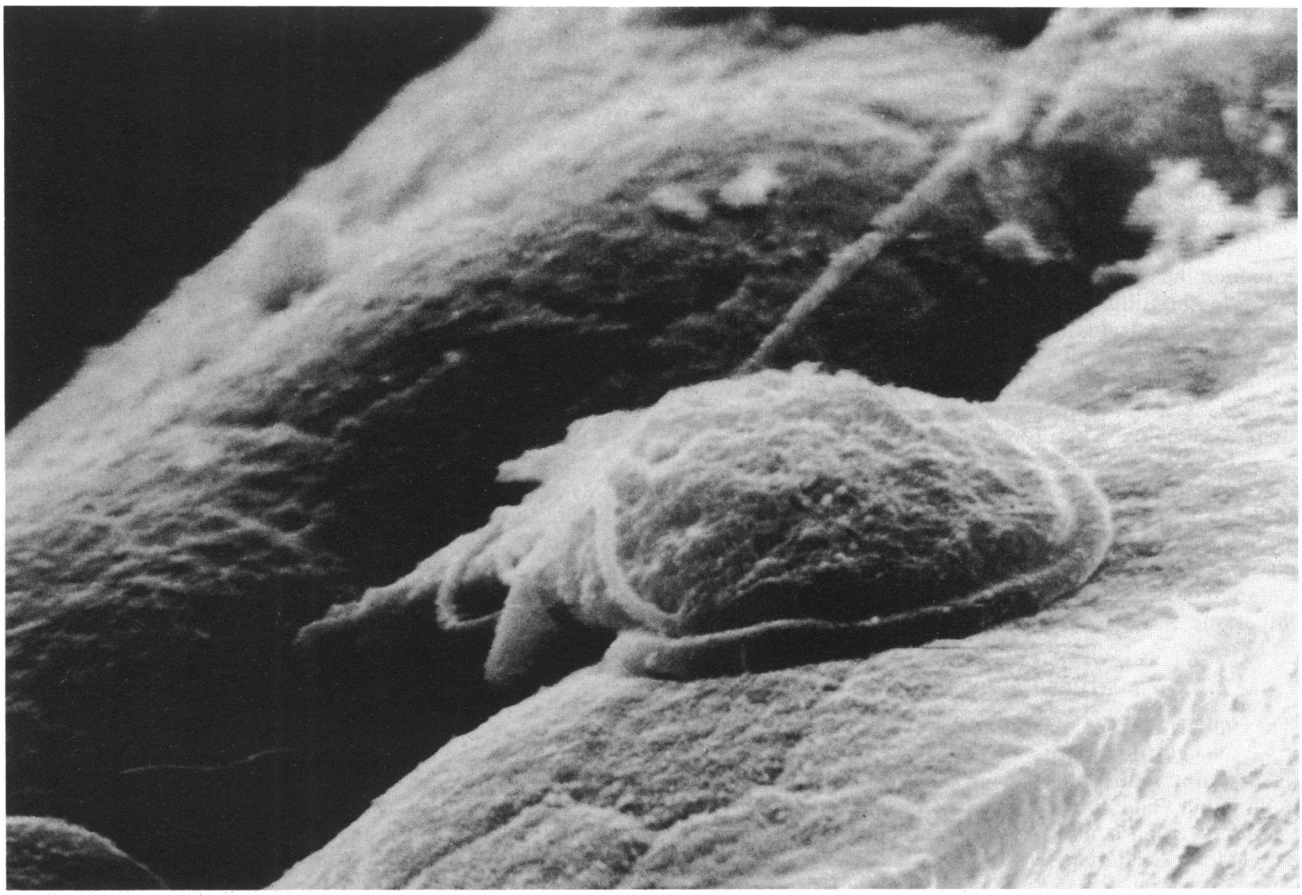


FIG. 7. Scanning electron micrograph of a *G. lamblia* trophozoite attached to the microvillous border of villus epithelial cells in a human jejunal biopsy. Reprinted with permission (95).

306). Indeed, growth with bacteria is essential for virulence of the pathogenic intestinal protozoan *Entamoeba histolytica* (265), so there is precedence for the idea. However, bacterial overgrowth is variably present, and in studies of infected human volunteers bacterial overgrowth was not associated with symptomatic giardiasis (240). In addition, treatment of bacterial overgrowth alone does not result in improvement of symptoms (17), suggesting that bacterial overgrowth is not related to the diarrhea of giardiasis. Disaccharidase deficiency is a common manifestation of giardiasis in humans (18) as well as in an animal model of *G. muris* infection (133). *G. lamblia* trophozoites demonstrated direct damage of mouse intestinal mucosal cells in vitro with decreased levels of disaccharidases (19), suggesting that the disaccharidase deficiency is a direct effect of giardiasis. Shortening or even flattening of the intestinal villi is common in patients (Fig. 6) and may be the cause of the diarrhea. Nude mice develop prolonged infection with *G. muris* and demonstrate some villus changes, but these changes are greater in nude mice reconstituted with lymphoid cells (270). Another study demonstrated no clear difference between nude and immunocompetent mice in the degree of villus changes (113). Therefore, the *Giardia* trophozoite appears to directly cause at least part of the villus changes, which may be exacerbated by an effective immune response. The possibility of an enterotoxin has been suggested, but none has been described to date. Further studies of the interaction between *Giardia* spp. and the intestine, including the effect of the surface antigens on the mucosa and epithelial cells, are necessary.

IMMUNE RESPONSE

An evaluation of the host immune response to *Giardia* infection must consider the relatively long duration of infection and the apparent development of partial resistance to reinfection. Our knowledge of the host immune response to *Giardia* infection comes from several sources. The following have been the most important: (i) clinical and epidemiologic studies of infected humans; (ii) prospective analysis of experimentally infected human volunteers; (iii) in vitro studies with axenically grown *G. lamblia* trophozoites and immune cells from a variety of hosts; (iv) an animal model involving *G. lamblia*-infected gerbils; and (v) an animal model involving *G. muris*-infected mice. In addition, a few studies from India have used *G. lamblia*-infected mice. Interpretation of many of the in vitro studies is problematic because *G. lamblia* trophozoites do not survive well in medium used for cell culture (discussed below) and indirect measures of trophozoite survival were used. The use of more direct measures of trophozoite survival (158) and the use of RPMI 1640 with 11.4 mM cysteine (143) may increase the reliability of the in vitro studies.

The best source of information regarding the host immune

has been the murine model of *G. muris* infection, first described in 1976 (271). In the initial description, *G. muris* cysts were inoculated into the esophagus of Swiss albino mice, resulting in an intestinal infection that reached a maximum trophozoite count in 1 to 2 weeks and resolved spontaneously in 3 to 4 weeks. Infected mice demonstrated impaired weight gain, and histologic examination of the jejunum revealed villus flattening. Since then, a number of mouse strains with various immune defects have been studied; they are discussed below. The advantages of this model are the similarity of illness seen in *G. muris*-infected mice and *G. lamblia*-infected humans, as well as the variety of defined mouse strains available and our knowledge of the mouse immune system. The major disadvantages are that adult mice cannot routinely be infected with human *G. lamblia* isolates and that *G. muris* isolates have not been grown axenically. Therefore, the various human isolates cannot be compared in the mouse model. For this reason, other animal models have been developed, and the most useful has been the *G. lamblia*-infected gerbil (32). The *G. lamblia*-infected gerbil develops an illness similar to that of the *G. muris*-infected mouse, and, like the mouse, the gerbil spontaneously clears its infection and is partially resistant to reinfection.

Humoral Immunity

The importance of the humoral immune response was initially suggested by the occurrence of severe and prolonged symptomatic giardiasis in patients with hypogammaglobulinemia. The activity of anti-*Giardia* antibody against *G. lamblia* trophozoites in vitro has been documented in several studies. Human serum from patients with no history of giardiasis and a negative indirect fluorescent-antibody assay killed between 8 and 76% of trophozoites from the P-1 isolate, and serum with an anti-*Giardia* indirect fluorescent-antibody assay titer of 1:128 from a patient with a recent history of giardiasis killed 98% of the trophozoites (156). The killing was dependent on the classical complement pathway. Other patterns of antibody-mediated killing have also been identified. For example, an IgG monoclonal antibody to a 170-kDa cysteine-rich variant surface antigen (CRP 170) demonstrates complement-independent cytotoxicity for trophozoites expressing CRP 170 (233, 234). However, monoclonal antibodies and rabbit antiserum directed against other variants of the cysteine-rich surface antigens typically demonstrate complement-dependent cytotoxicity (234). In other studies, *G. lamblia* WB trophozoites were lysed by IgM in the presence of complement (74). C2 and C4 (classical pathway only) were not needed; however, C1 components and calcium (classical pathway) were required. Partial lysis was seen without C9, and full lysis was seen with C9. Therefore, different requirements of complement for antibody-mediated killing have been noted depending on the exact antibody or antigen. The relevance of complement in resolution of the actual infection must be questioned, because complement levels in the intestine are low (212). In addition, secretory IgA is probably the most important component of the antibody response and does not use complement for microbial toxicity (185).

An intestinal response followed by a serum IgA response has been documented in mice infected with *G. muris* (295). The appearance of IgA in the intestinal secretions correlated with resolution of infection. Immunohistologic studies of the Peyer patches of *G. muris*-infected mice (53) demonstrated that 65% of the cells were B cells (30% IgA and 52% IgM), with a peak IgA response at 11 to 14 days. In other studies, the intestinal antibody responses of BALB/c and nude mice to *G. muris* infection have been compared (151, 270, 308). In a detailed analysis of the antibody response (151), 31 and 18% of trophozoites were coated with IgA and IgG, respectively, in the BALB/c mice and very few were coated in the nude mice. No IgM response was noted in either strain. The efficacy of passive transfer of antibody was suggested by the ability of human milk from *Giardia*-infected mothers to protect neonatal mice from *G. muris* infection whereas milk from nonimmune mothers showed no protection (21). The milk from immune mothers contained *Giardia*-specific IgG and IgA, but not IgM. A biliary IgA antibody response has also been documented in *G. lamblia*-infected rats, in which IgA antibodies coated the surface of the trophozoite (207).

Treatment of two strains of mice with rabbit anti-IgM from the time of birth effectively suppressed total and *Giardia*-specific serum and secretory immunoglobulin (IgG, IgA, and IgM) responses and resulted in persistence of *Giardia* infections in the anti-IgM-treated mice but not in controls (293). In other studies, *G. muris* infection was chronic in *xid* mice but not in crosses between BALB/c and *xid* mice that do not express the *xid* gene (294), despite normal serum and intestinal IgA responses. Mice expressing the *xid* gene have a defect in B cells, and the interpretation of the authors was that the *xid* mice were probably deficient in a critical specific IgA antibody.

In studies of the *Giardia*-specific immune response in infected human volunteers, serum IgM (100% of patients), IgG (70%), and IgA (60%) and intestinal IgA (50%) were documented (240), but in these patients the IgA response was not clearly correlated with resolution of infection. However, a clinical study from India corroborated in humans the finding of the protective effect in animals of antibody passively acquired from milk. In this study, mothers with giardiasis had anti-*Giardia* secretory IgA antibodies in their milk (244). Their infants had a lower incidence of giardiasis than infants born to mothers without giardiasis, suggesting a protective role for passively acquired antibody in humans.

T Lymphocytes

Lymphocyte proliferation is seen in mice infected with *G. muris* and is correlated with the resolution of infection (52, 155). The total number of T cells in the Peyer patches doubles, but the CD4-to-CD8 ratio remains unchanged (52). Nude (athymic) mice develop chronic giardiasis (154, 270), suggesting that the T cell plays an important role in elimination of infection. In addition, BALB/c mice depleted of CD4 (L3T4) lymphocytes with anti-lymphocyte monoclonal antibodies demonstrate defective clearing of *Giardia* infection, whereas those depleted of CD8 (Ly+2) cells clear their infection normally (152). The observation that corticosteroid-treated (231) or cyclosporin A-treated (31) mice with *G. muris* infection have increased numbers of organisms also suggests the importance of T-cell immunity, since these agents primarily suppress T-cell function. Recrudescence of infection has been observed in *G. lamblia*-infected gerbils after corticosteroid treatment (202), suggesting not only the importance of T-cell-mediated immunity, but also the possibility that latent *Giardia* infection is common. Therefore, the cell-mediated immune response appears to be important in the animal model of *G. muris* infection. Most probably, this is mediated through a T-cell-dependent antibody response to the infection.

Chronic or severe giardiasis in humans has not been associated with defects in T-cell-mediated immunity, but antibody response to *G. lamblia* infection is depressed in patients with AIDS (167). Whether there is a difference between human and murine giardiasis in the importance of the cellular immune response is unknown.

Macrophages

A study showing spontaneous cytotoxicity of macrophages from noninfected humans for *G. lamblia* trophozoites (289) suggested the importance of the macrophage in the immune response to *Giardia* infection. However, this study was later refuted by one of the original authors (9) because of methodological problems in the initial study. In the initial experiment, trophozoites were radiolabeled and killing was measured by the release of radioactivity in the presence of the effector cell. During the long (16-h) incubation, *Giardia* trophozoites died in the absence of an adequate cysteine concentration and radioactivity was released when the effector cell phagocytosed dead trophozoites. Therefore, there is no conclusive evidence that nonimmune macrophages ingest and kill *Giardia* trophozoites in the absence of anti-*Giardia* antibody.

The same methodological difficulties have also plagued other studies showing cytotoxicity of macrophages (180) and lymphocytes (179) and antibody-dependent cytotoxicity of neutrophils (292) for *Giardia* trophozoites. A cysteine concentration high enough to support in vitro growth of *G. lamblia* is toxic to human macrophages (1), making it difficult to do prolonged (more than several hours) cytotoxicity studies. In contrast, RPMI 1640 supplemented with 11.4 mM cysteine will support survival and attachment of *G. lamblia* trophozoites as well as lymphocyte survival and proliferation for at least 6 hours at 37°C (143). Therefore, cysteine supplementation of RPMI 1640 may facilitate at least some in vitro investigations of the interaction between immune cells and *G. lamblia* trophozoites.

In contrast to the studies with nonimmune macrophages, several studies have demonstrated phagocytosis of *Giardia* trophozoites by macrophages of infected hosts. Intestinal macrophages from mice infected with *G. muris* have been shown in histologic studies to phagocytose trophozoites (249). In vitro phagocytosis and killing of *G. muris* trophozoites by intestinal macrophages are markedly enhanced by immune rabbit serum and by immune milk (181). Peritoneal macrophages from reinfected mice demonstrate slightly greater phagocytosis for *G. lamblia* trophozoites than do those from initially infected mice (180). Similarly, ingestion and killing of *G. lamblia* trophozoites by human macrophages were enhanced by heat-inactivated autologous immune serum (158). This study demonstrated microscopic evidence of phagocytosis within 2 h and documented the survival of trophozoites incubated in the same medium but without macrophages, thereby avoiding the problems of the above-mentioned studies.

One study compared two strains of mice, one susceptible and the other resistant to *G. muris* infection, and found that the macrophage response was greater in the resistant mice but did not demonstrate a cause-and-effect relationship (29). Other studies have compared intestinal macrophage function in mice with normal and defective clearing of *Giardia* spp. and found little difference in the degree of phagocytosis by macrophages (154, 249). The available evidence suggests that the macrophage does not spontaneously eliminate *Giardia* spp., but leaves open the possibility that it acts as an antigen-presenting cell for CD4 lymphocytes and/or participates in antibody-mediated killing of the trophozoites.

Other Immune Mechanisms

The neutrophil has been demonstrated to attach to *G. lamblia* trophozoites (181), but a polymorphonuclear infiltrate does not occur in giardiasis, and there is no evidence to suggest that the neutrophil plays an important role in elimination of *Giardia* infection. A report suggesting antibody-dependent killing of trophozoites by polymorphonuclear leukocytes (292) had the same methodological problems as the studies demonstrating activity of nonimmune macrophages.

Mast cell-deficient mice develop chronic *G. muris* infections (98), but the role of the mast cell has not been clarified. Other mouse strains also develop chronic giardiasis (e.g., C3H/He and A/J) (33, 308), but the defect(s) leading to chronicity is not well characterized. A strain of mice deficient in natural killer cells clears *G. muris* infection normally (153). The possibility that *Giardia* infection leads to partial immune suppression is suggested by the finding that *G. muris*-infected mice demonstrate reduced immune response to sheep erythrocytes (30). The suppression was transferable by mesenteric lymph nodes but not by spleen cells.

Surface Antigens and Antigenic Variation

Elucidation of the *Giardia* antigens important in the immune response to infection has been difficult for a variety of reasons: (i) protective immunity is only partial, making evaluation of protective immunity more complicated; (ii) asymptomatic infections are common; (iii) at least one major immunogen is variable among different isolates and demonstrates variation within a single cloned isolate; and (iv) investigators have used different isolates, different antibody reagents, and different means of studying surface antigens, making comparison of the results from different laboratories difficult. Nevertheless, I will attempt to analyze the current state of knowledge of the surface antigens of *G. lamblia* and offer a basis for future studies.

Surface iodination of the WB isolate with Iodogen or lactoperoxidase revealed an antigen that showed a smear from 94 to 225 kDa on polyacrylamide gel electrophoresis (PAGE) (237). This antigen was released into the medium in large quantities and was degraded by protease VI and periodate, but was resistant to trypsin and chymotrypsin. Hydrophobicity was suggested by adherence to phenyl-Sepharose, and lectin-binding studies (including WGA) revealed no carbohydrate groups. A similar antigen from the P-1 isolate was antigenically distinct. Further studies of the same antigen from 19 isolates (including WB and P-1) showed marked size and antigenic differences among isolates for this major surface antigen (241). When PAGE was performed without a reducing agent, the antigen migrated as a distinct band. Now, the WB antigen migrated at 170 kDa whereas others had variable numbers of bands ranging in size from 43 to 200 kDa. A cytotoxic monoclonal antibody, 6E7, specific for the 170-kDa antigen of the WB and other similar isolates (233) was used to demonstrate variation of this antigen within individual clones of the WB isolate (234). The size of the surface antigen repertoire for the WB isolate has been estimated to be between 20 and 184. In cloned WB and GS trophozoites, specific variants can be detected after approximately 12 and 6.5 generations, respectively (235).

A portion of the gene for the 170-kDa surface antigen from

WB was cloned and found to encode a cysteine-rich (12%) protein (called CRP 170) (2). When trophozoites expressing CRP 170 was metabolically labeled with cysteine, most of the label was incorporated into a 170-kDa doublet, and a 64-kDa doublet was labeled on the variant expressing the 64-kDa antigen. Immunoprecipitation was used to confirm that these cysteine-labeled doublets reacted with the respective monoclonal antibodies. The reason for a doublet is unknown, but it may represent some type of protein processing. Metabolic labeling has also been used to confirm the presence of cysteine-rich proteins on other isolates (8).

When Southern blots of DNA from a number of WB clones were probed with the cloned portion of the CRP 170 gene, numerous rearrangements were identified, but could not be clearly associated with the presence or absence of expression of CRP 170 (2). The presence of multiple bands of hybridization identified it as a multigene family. Therefore, the mechanism of antigenic change is not known, but may depend on DNA rearrangements. Since then the genes for other cysteine-rich proteins have been cloned: TSA 417 (126), VSP 1267 (228), and CRP 72 (5). Each has a cysteine content of 11 to 12%, and each is variable within single isolates. The 3′ C-terminal amino acid sequences are highly conserved among these proteins. Antibodies to TSA 417 react with the entire surface of live trophozoite, prevent attachment, and immunoprecipitate the major antigens from surface-labeled trophozoites. Interestingly, surface-labeled trophozoites and *Escherichia coli* expressing the recombinant protein expressed 66- and 85-kDa polypeptides, suggesting protein processing or a conformational change. The reason for the discrepancy with the predicted size of 72.5 kDa is unknown.

Other surface antigens have been identified in other studies by surface iodination and may be part of the family of cysteine-rich proteins, but cysteine labeling and sequence analysis of the gene were not performed, so that the relatedness cannot be established or refuted. For example, an 82-kDa antigen was identified on the WB, P-1, LT, and RS isolates by surface iodination and Western blotting. It was hydrophobic and partially sensitive to pronase and periodate (90). Surface iodination and immunoprecipitation with a monoclonal antibody and human antiserum were used to identify an 88-kDa antigen on the P-1 isolate (87). The monoclonal antibody reacted diffusely with the surface and flagella and with the WB isolate. Isolated membranes from surface-iodinated trophozoites of the P-1 isolate showed a broad band 70 to 80 kDa in size (59).

The presence of a number of surface glycoproteins on the surface of *G. lamblia* trophozoites has been proposed on the basis of binding to the lectin WGA (157, 247, 322). In studies with WB trophozoites grown in TYI-S-33 with bile and harvested in the late log phase, the binding of WGA to trophozoites was abolished by treatment with *N*-acetyl-β-D-glucosaminidase; therefore, the carbohydrate-binding moiety was assumed to be GlcNAc (322). Later experiments with the P-1 isolate confirmed the presence of GlcNAc on trophozoites and in vitro-derived cysts by using gas chromatography, mass spectrometry, and specific antibodies for GlcNAc (247). A number of sizes of proteins were identified by probing a Western blot of trophozoite proteins with WGA. When whole trophozoites were labeled with fluorescent tagged WGA, no reaction was seen with 10 to 20% of the organisms. The authors proposed the possibility that these unlabeled organisms represented encysting cells. Other investigators did not find GlcNAc on P-1 trophozoites grown without bile (171) and have proposed that GlcNAc is an intermediate formed during GalNAc synthesis during the process of encystation (168). Further investigation with trophozoites grown with and without bile may be required to address these different results.

A 65-kDa protein has been identified in WB trophozoites and in cysts from the feces of infected humans (276, 277). The protein bound to the lectin castor bean agglutinin-peroxidase (RCA-I), and the binding was inhibited by galactose, suggesting the possibility that the 65-kDa protein was glycosylated. No binding to WGA was observed.

A 49-kDa glycoprotein (GP49) is present on the surface of trophozoites from various geographic locations and, in contrast to the cysteine-rich protein, TSA 417, has not shown variation (218). In metabolic labeling studies with radiolabeled palmitic and myristic acids, most of the label was incorporated into the 49-kDa glycoprotein and further experiments demonstrated that GP49 had a glycosylphosphatidylinositol anchor (72).

A study of the human response to specific *Giardia* antigens (301) revealed that a number of antigens, including a prominent response to a 31-kDa antigen (possibly giardin) were recognized by some patients, but the role of the 31-kDa or other antigens in resolution of infection could not be evaluated. The importance of the family of cysteine-rich proteins is demonstrated by the marked susceptibility of trophozoites to antibodies directed against these proteins, but their role in the biology of the organism and in pathogenesis is unknown. Similarly, the large repertoire of cysteine-rich proteins and the rapid change in expression suggest the biological importance of antigenic variation, but its specific role is unknown. A variety of other organisms also have the ability to change the expression of their surface proteins, apparently for a variety of reasons.

African trypanosomes are protozoans that cause African sleeping sickness. The illness is characterized by waves of parasitemia followed by an effective host immune response that nearly eliminates the infection. However, a few organisms survive DNA rearrangements that lead to expression of a variant surface glycoprotein that is structurally similar to, but antigenically very different from, the previous variant surface glycoprotein (78). Therefore, the parasite begins to multiply again. The bacterial pathogens *Borrelia hermsii* (214) and *Neisseria gonorrhoeae* (299) also exhibit extensive repertoires of variant surface antigens. Evasion of the immune response is the most likely purpose of antigenic variation in each of these pathogenic organisms. The free-living ciliated protozoa *Paramecium* spp. also have a variant surface protein, called the i-antigen. This protein is large (>200 kDa) and cysteine rich (11%) (262) and varies in response to environmental changes such as temperature, pH, and composition of the culture medium (263). Unlike the above organisms, an entire population can change expression; the change is apparently induced rather than selected. The role of this surface protein and the reason for its variation are unknown, but the change is not for the purpose of evading an immune response, since *Paramecium* spp. are free-living organisms.

Antigenic variation of *G. lamblia* in a host has been demonstrated in gerbils (12), experimentally infected humans (239), and neonatal mice (141) and is a possible explanation for the chronicity of giardiasis despite the susceptibility of *G. lamblia* to antibody-mediated killing. However, in the gerbil model a change of the surface antigen was documented within 7 days after inoculation and no further changes were noted between 7 and 28 days. This temporal pattern is more consistent with an initial adaptation to the

host from the axenic growth medium than with a response to host immunity. Similarly, neonatal mice were infected with a cloned GS isolate expressing a 72-kDa major surface antigen (141). The mice recovered spontaneously by day 42, and the 72-kDa antigen was no longer present by day 22. A T-lymphocyte-dependent immune response to the original 72-kDa antigen was not present. Therefore, the original surface antigen evoked either a T-cell-independent response or no immune response. In human volunteers infected with the same GS clone, antibody to the 72-kDa surface antigen was present at 22 days, when the 72-kDa antigen was no longer detectable (239). The temporal pattern of antibody response in humans is consistent with the hypothesis that antigenic variation is a means of evading the host response.

An intriguing alternative reason for variation of the cysteine-rich proteins is suggested by the finding that organisms expressing different cysteine-rich proteins are variably susceptible to proteases (243). Trypsin and chymotrypsin were cytotoxic for WB trophozoites expressing CRP 170, but some organisms survived and, when expanded, were stably resistant to these intestinal proteases and expressed different surface antigens. In contrast, the GS isolate, which was virulent in experimental human infections, was initially resistant to trypsin and chymotrypsin. Perhaps different cysteine-rich proteins allow the trophozoite to survive in different intestinal milieus and thereby to infect different hosts.

Nonimmune Protection

Nonimmune mechanisms of defense against *Giardia* infection are probably important also. Nonimmune human milk is able to kill *G. lamblia* trophozoites through digestion of milk triglycerides by a bile salt-stimulated lipase, releasing free fatty acids that are toxic to *G. lamblia* trophozoites (131, 149, 268, 272). The killing is unrelated to secretory antibody and is not found with nonhuman milk. The observation that *Giardia* spp. very rarely invade the intestinal wall suggests the importance of the mucus lining, and the importance of intestinal motility is suggested by the demonstration that gerbils infected with *G. lamblia* are more resistant to infection when they are fed a high-fiber diet (199).

Summary of Immunity

In summary, the humoral immune response is critical for elimination of infection, both in humans and in animal models of giardiasis. It is likely, but not proven, that secretory IgA is the most important component of the humoral immune response. In *G. muris*-infected mice, the antibody response is dependent on intact cell-mediated (CD4) immunity, but whether the antibody response in humans is T-cell dependent is unknown. The macrophage may function as as antigen-presenting cell and/or may phagocytose and kill opsonized trophozoites. Important forms of nonimmune protection include the intestinal mucus layer, intestinal motility, and human breast milk for infants. It is possible that antigenic variation and the *Giardia* IgA protease are used by the organism in evading the immune response.

DIAGNOSIS

Microscopic examination of the stool for cysts and trophozoites is usually the first diagnostic test performed in patients with suspected giardiasis. *Giardia* cysts are found in the stools of most patients with giardiasis (137), whereas trophozoites are found less commonly, but correlate with symptomatic infection. Stool specimens are examined by light microscopy either fresh or fixed with polyvinyl alcohol or formalin and then stained with trichrome or iron hematoxylin (288). Stool specimens may be concentrated by the formalin-ethyl acetate or zinc sulfate methods. The cysts can also be detected by immunofluorescent antibody labeling (Meridian Diagnostics, Cincinnati, Ohio) (298). The sensitivity of routine examination of a single stool specimen for cysts is approximately 50 to 70%, so two or three specimens collected on different days should be analyzed (150). In addition, symptoms of illness may begin approximately 1 day (240) to 1 week (174) before excretion of cysts begins, so the stool examination should be repeated for patients with persistent symptoms in whom a diagnosis has not been established.

In some patients with chronic diarrhea, stool examinations are negative despite the presence of trophozoites in the duodenum. The diagnosis of giardiasis can be established in these patients by examination of duodenal contents. The easiest method is to have the patient swallow a gelatin capsule on a string (Entero-Test; Hedeco Corp, Mountain View, Calif.) (25). The capsule remains in the intestine at least several hours and is then removed and examined microscopically. Duodenoscopy with microscopic examination of duodenal fluid or histologic examination of biopsy specimens is sometimes necessary. Although more invasive, it has the advantage of addressing other diagnostic possibilities. Some studies have suggested that examination of duodenal contents is more sensitive than examination of stool specimens (178, 274), but another study demonstrated greater sensitivity for examination of stool specimens (137). Cysts were found in the stools of all 10 experimentally infected persons, while duodenal aspiration was uniformly negative (240). Therefore, evaluation of stool and duodenal contents is complementary, and one test may be positive while the other is negative.

Detection of *Giardia* antigens by counterimmunoelectrophoresis (64, 312) or enzyme-linked immunosorbent assay, using whole antibody to whole trophozoites (138, 142, 238, 309) or monospecific antibodies to GSA65 (275) in stool specimens, is reported to be more sensitive than detection of cysts and is less labor intensive than microscopic examination. Serologic testing is useful epidemiologically, but is not sensitive or specific enough for diagnosis of individual patients (136, 290, 313).

TREATMENT

A number of effective agents, including quinacrine, the nitroimidazoles metronidazole and tinidazole, and furazolidone are available for treatment of patients with giardiasis (Table 2) (73). Metronidazole has no effect on cyst viability, whereas quinacrine has some effect (251). However, it is unknown whether cysts produced in the small intestine then have the ability to excyst in the same host and continue the infection, and whether the major therapeutic effort is therefore directed against the trophozoite.

Quinacrine is an antimalarial agent that is highly effective for treatment of giardiasis when given for a 5- to 10-day course (73) and is considered by some to be the agent of choice. However, gastrointestinal side effects are common, sometimes causing difficulty with compliance (230). Rare side effects include toxic psychosis and hemolysis in glucose-6-phosphate dehydrogenase-deficient patients. The mecha-

TABLE 2. Commonly used treatment regimens for giardiasis

Agent	Daily dose (frequency)	Duration	Efficacy[a] (reference)
Quinacrine	6 mg/kg/day (three times daily), 300 mg/day maximum	5 days	>90% (73)
Metronidazole	15 mg/kg/day (three times daily), 750 mg/day maximum	5 days	>90% (73)
Tinidazole[b]	50 mg/kg, 2 g (maximum)	Single dose	>95% (176,297)
Furazolidone	8 mg/kg/day (three or four times daily), 400 mg/day maximum	10 days	>80% (73)
Paromomycin[c]	30 mg/kg/day (three or four times daily)	10 days	Unknown (54)

[a] Many of the comparative trials are reviewed in reference 73.
[b] Not available in the United States.
[c] Its efficacy is probably lower than that of the other agents, but it is sometimes recommended for pregnant women because it is poorly absorbed.

nism of action of quinacrine is possibly due to its effect on the flavoprotein and quinone components of respiration (251).

The nitroimidazoles metronidazole and tinidazole are also highly effective for the treatment of giardiasis. They have a broad spectrum of activity against anaerobic bacteria and protozoans as a result of their reduction to nitro anion radical metabolites through ferredoxin or ferredoxinlike molecules (115, 227). The radicals or an intermediate, such as hydroxylamine, then bind to DNA or protein molecules (165). Although the interaction with DNA has been proposed as an important component of the effect of metronidazole (165), the rapid inhibition of respiration in *G. muris* trophozoites (251) suggests that the radicals may have a toxic effect on the enzyme(s) of the respiratory pathway. Metronidazole is widely used in the United States for treatment of giardiasis (although not approved by the Food and Drug Administration for treatment of giardiasis) and is more than 90% effective when given for a 5-day course. Nausea and general malaise are common during therapy, and a disulfiramlike interaction with ethanol can be seen, but serious side effects are rare. Metronidazole is mutagenic in bacteria (198, 273) and, in high doses for prolonged periods, is carcinogenic in mice (279), so concerns have been raised about its use in humans. However, two case-control studies have shown no increased frequency of cancer in people who have taken metronidazole (26, 27).

Tinidazole is effective when given as a single dose and is very well tolerated (176, 297). For these reasons, it is probably the treatment of choice in countries where it is available; however, at the time of writing, it has not been approved in the United States.

Furazolidone is frequently used in children because the bitter taste and gastrointestinal side effects of quinacrine make the latter difficult to use, but furazolidone may be somewhat less effective than quinacrine and metronidazole (73). It most probably acts by inhibiting anaerobic respiration (70, 88), but it also binds to DNA. Like metronidazole, it is carcinogenic in animals (rats), but has not been shown to be carcinogenic in humans.

Paromomycin is a nonabsorbable aminoglycoside that has some in vitro activity against *G. lamblia* (80). Aminoglycosides inhibit protein synthesis through binding the small-subunit (16S-like) rRNA (80). The sequence of the DNA encoding the small-subunit rRNA was used to predict susceptibility to paromomycin, but not to many of the other available aminoglycosides, and the prediction was confirmed by in vitro testing. Clinical data regarding the efficacy of paromomycin are very limited (54, 192), but it is frequently recommended for treatment of symptomatic giardiasis during pregnancy because of concerns about possible teratogenic effects of the other available agents (278).

Mebendazole, a benzimidazole, is a broad-spectrum antihelminthic agent that probably works through its interaction with β-tubulin. It demonstrates in vitro activity against *G. lamblia* (84), and in one uncontrolled clinical trial with a dose of 200 mg/kg/day, 38 of 40 patients (95%) were cured (16). The duration of therapy was not specified, but was apparently at least 5 days. In another study, the same dose given for 1 day was ineffective (121). Therefore, mebendazole is probably effective for treatment of giardiasis, but at much higher doses than used for helminthic infections (184). The benzimidazole albendazole also has in vitro activity against *G. lamblia* (217) and was effective in five patients (332). Controlled trials are necessary to compare the safety and efficacy of the benzimidazoles with those of the standard agents.

Other agents with in vitro activity include chloroquine (140), pyrimethamine (140), mefloquine (70), rifampin (70), azithromycin (70), and the lipophilic tetracyclines, such as doxycycline (70, 81), but clinical studies have not been performed.

Patients who fail to respond to treatment usually respond to a second course of treatment with the original or another agent. Decreased in vitro susceptibility to metronidazole (44) and furazolidone (213) has been documented, but has not been clearly correlated with treatment failure, and high-grade in vitro resistance to these agents has not been reported. However, it is difficult to culture *Giardia* spp. in samples from patients, and in vitro testing for susceptibility is not standardized. Therefore, in vitro susceptibility is difficult to evaluate in an individual patient, and so true drug resistance is difficult to document. Combined treatment with quinacrine and metronidazole has been used successfully in infections that were refractory to treatment with a single agent (213, 291, 302).

Treatment of humans who have asymptomatic giardiasis is controversial, and the decision is made largely on the basis of public health issues (i.e., the risk of transmission to other individuals). In highly endemic areas, the rate of reinfection after treatment is so high that treatment of asymptomatic patients is futile (135). On the other hand, symptomatic giardiasis can clearly result after exposure to persons with asymptomatic infection (248). Therefore, in areas where the risk of reinfection is lower, people with asymptomatic infection should probably be treated to prevent transmission to others.

CONCLUSIONS

G. lamblia was described more than three centuries ago, but has only recently become generally recognized as a human pathogen. Our knowledge of *Giardia* spp. has greatly increased since 1976, the year when *G. lamblia* was first axenized and when the murine model of giardiasis was described. *G. lamblia* has been proposed as the most prim-

itive eukaryotic organism and is one of the few eukaryotes with an anaerobic metabolism and a total dependence on salvage of exogenous nucleotides. Other important findings in the last 15 years include documentation of antigenic variation and an improved understanding of the host immune response. Completion of the life cycle in vitro makes *G. lamblia* a potential model for studying developmental changes. The small rRNA and the short 5′ leader sequences on mRNA suggest that the minimum requirements for translation initiation in eukaryotes can be better understood from studying the mechanism in *Giardia* spp. Other unique features of *Giardia* spp. include the ventral sucking disk used for attachment and the presence of two apparently identical nuclei.

Some of the areas of investigation that promise to be especially interesting and fruitful are as follows. (i) Accurate species and strain definition, with determination of the extent of cross-transmission between different hosts, differences seen at the electron-microscopic level, as well as DNA comparisons (e.g., rDNA sequences), should facilitate accurate epidemiologic investigations. (ii) Genomic organization, including ploidy, number of chromosomes, significance of chromosome plasticity, and question of a sexual cycle. What is the role of the two nuclei? (iii) Mechanism of protein translation initiation in *Giardia* spp. The very short 5′ untranslated RNA sequence suggests the possibility that translation initiation in *Giardia* spp. is different from that in higher eukaryotes. (iv) Characterization of the metabolic enzymes and pathways. In addition to its importance as a primitive eukaryote, the unique enzymes and pathways in nucleotide salvage and energy metabolism may provide further avenues for chemotherapeutic intervention. (v) Determination of the mechanism of adherence. Do trophozoites attach to the intestinal wall strictly by mechanical suction, or are other mechanisms, such as receptor-mediated attachment, also involved? Specific inhibitors of proteins involved in attachment and motility, such as giardin and tubulin, may be useful in the chemotherapy of giardiasis. For example, mebendazole, a tubulin inhibitor, has shown promise for treatment of giardiasis. (vi) Elucidation of the mechanism of encystation and excystation. The ability to complete the life cycle in vitro may aid our understanding of this important process in *Giardia* spp. as well as providing clues for understanding the regulation of life cycles in other organisms. (vii) Further understanding of antigenic variation (its purpose, mechanism, and the role of the family of cysteine-rich surface antigens). (viii) Mechanism(s) of pathogenesis. Is diarrhea produced by a toxin-mediated effect, by covering the intestinal epithelium, or by other means? (ix) Analysis of the intestinal immune response to giardiasis, including the role of the T lymphocyte and macrophage, and the events leading to isotype switching in the intestine.

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